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(54) Title: STEATOSIS-MODULATING FACTORS AND USES THEREOF

(57) Abstract: The present invention relates to a method of modulating the muscular steatosis-modulating factors (MSMF). The determination of concentrations of the MSMF is described for the establishment of the steatotic state in individuals. Also, is disclosed a method of selecting individuals to serve as founders of animal lineages. The present method involved too the treatment of human and animals with agonists or antagonists of MSMF depending of the effects desired.

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STEATOSIS-MODULATING FACTORS AND USES THEREOF

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to a measurement of the level of muscular steatosis-modulating factor (MSMF) in human or animal. The method is performed by measuring level of MSMF in a biological sample, and then screening individual having normal and abnormal level of MSMF.

(b) Description of Prior Art

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Mammalian skeletal muscle normally undergoes a reparative process after oxidative stress or traumatic injury. The process of skeletal muscle repair is actually a series of discrete overlapping events, which can be segregated into trauma, tissue degeneration, inflammation, phagocytosis, angiogenesis, stem cell activation, migration of the stem cells to the site of injury, proliferation of undifferentiated stem cells, re-innervation, differentiation of the stem cells, and remodeling of the tissue.

The early restored muscle tissues approximate embryonic-like satellite cells containing centrally located nuclei and lies adjacent to mature myofibers containing peripherally located nuclei. Unfortunately, restoration of physiological function may be compromised due to the increased proliferative nature of the surrounding connective tissues, eventually forming non-functional scar tissue.

Research in other areas has indicated that various factors such as platelet derived growth factor (PDGF), chicken muscle growth factor (CMGF), epidermal growth factor (EGF), sciatic nerve extract, insulin,

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and somatomedins stimulate a mitogenic or proliferative response in cultured muscle cells. This response should be contrasted with a myogenic response that does not induce myogenic lineage commitment of uncommitted stem cells, but instead induces the lineage commitment of the stem cells.

Three growth factors, insulin and insulin-like growth factors, namely insulin-like growth factor-I (IGF-I), also called somatomedin-C, insulin-like growth factor-II (IGF-II), also called myogenic stimulating activity, have been shown to be potent stimulators of skeletal muscle cell growth and differentiation in cultured myosatellite cells and myogenic lineage-committed stem cells by Ewton and Florini, Dev. Biol. 83:31-39 (1981); Florini et al., J. Biol. Chem. 261:16509-16515 (1986); Sejersen et al., Proc. Natl. Acad. Sci. 83:6844-6848 (1986).

Several in vivo studies have employed basicfibroblast growth factor (FGF-2) also named FGF-2, (TGF-beta), transforming growth factor beta epidermal growth factor (EGF) to stimulate internal wound healing. Buckley et al., Proc. Natl. Acad. Sci. 82:7340-7344 (1985); and Roberts et al., Proc. Natl. Acad. Sci. 83:4167-4171 (1986)noted that administration of FGF-2, TGF-beta, and EGF appeared to promote proliferation of connective tissue elements to form scar tissue and thus aid in wound healing of mammalian skeletal muscle.

In vitro studies have demonstrated the influence of other growth factors on the resultant phenotypic expression in myogenic cultures. For example, Hauschka (Lim and Hauschka, J. Cell Biol. 98:739-747 (1984); and Olwin and Hauschka, Biochemistry 25:3487-3492 (1986))

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and co-workers have reported that acidic-fibroblast growth factor (aFGF) and basic-fibroblast growth factor (FGF-2) play a dual role in stimulating myoblast proliferation while directly repressing terminal differentiation, as described by Linkhart et al., Dev. Biol. 86:19-30 (1981).

Unfortunately, the administration of growth factors that inhibit terminal myogenic differentiation, promote myoblast proliferation, and promote fibroblast proliferation and differentiation as a method to promote muscle repair appears to cause an increase in connective tissue scar formation. In muscle, increased scar formation creates decreased physiological function. A decrease in connective tissue scar formation with a compensatory increase in skeletal muscle mass plus revascularization and re-innervation of the tissues is necessary for the restoration of physiological function.

Obesity has been declared a public health hazard by the National Institutes of Health. To combat this health problem, both prophylactic and therapeutic approaches are necessary. For prophylactic purposes, it would be useful to be able to predict and measure a person's propensity or susceptibility to obesity for therapeutic purposes, a means for interfering with the development or differentiation of adipocytes (fat cells) would be of great benefit. Furthermore, as a broader preventative approach to obesity, the ability to limit the fat content of food mammals would be of great importance. None of these desired objectives has been achieved. A weight reduction program cannot efficiently control early-onset obesity once the obesity is apparent. Therefore, a means for early

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detection of early-onset obesity is imperative for its prevention.

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It is held that excessive ingestion of fat and carbohydrate induces obesity and hyperlipidemia and even develops hypertension and arteriosclerosis ultimately. The desirability of repressing the absorption of fat and carbohydrate and diminishing the accumulation of fat has, therefore, been finding enthusiastic recognition.

Infants, on exposure to excessive ingestion of nutriments, suffer increase of adipocytes and assume the state that may well be called potential obesity. For this reason, it has been reported that the repression of the increase of the number of adipocytes particularly in infants results directly in the prevention of the obesity and the cardiovascular diseases which may well be called complications of obesity in children and consequently in adults.

For the therapy of obesity and hyperlipidemia, such measures as limitation of meal, ingestion of diet food (such as, for example, fibers), and even administration of various medicines have been in vogue. The medicines now in popular use include dextran sulfate which enhances the lipoprotein lipase activity in blood, Nicomol™ that inhibits absorption of lipid, especially cholesterol, and Clofibrate™ and Pravastatin™ which are agents for improving metabolism of lipid, for example.

Unfortunately, the limitation of meal is an agony for persons obliged to pursue this exercise and the administration of such medicines as mentioned above possibly entrains side effects.

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Replacement of myofibres by adipose cells, usually with no decrease in muscle volume is defined as muscular steatosis.

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Reports concerning muscular steatosis (MSt) in 5 animals is alternatively named progressive primary myopathy, pseudohypertrophic atrophy, lipomatous pseudohypertrophy, interstitial lipomatosis, lipomatous ' muscular dystrophy, myosclerosis, and hypoplasia or MSt is typically found atrophia lipomatosa. otherwise healthy cattle and pigs but it also occurs in 10 dogs, sheep, fish, birds and human. Cattle with MSt sometimes have an abnormal gait with hind feet knuckled over and erratic hind limb movements. Affected animals stand normally, but sway or stagger when blindfolded. Lesions are usually bilaterally symmetrical and may appear almost anywhere in the carcass, although longissimus dorsi and hind limb muscles are most frequently affected. Myofibres in affected areas may lack transverse striations and may be fragmented or vacuolated. Remaining myofibres may be hypertrophied, possibly a compensatory mechanism, or atrophied with an increase in number of nuclei. An important feature is that there is inflammatory cells usually invade no evidence of myofibre regeneration in MSt. Areas of MSt. Proliferation, or replacement by adipose cells is a 25 common finding in many myopathies, especially terminal cases, and does not necessarily indicate MSt.

Muscles of meat animals, especially at market weight, contain large numbers of adipose cells that play a major role in the determination of meat quality. Since adipose tissue is normally found intramuscularly, MSt must be viewed in the context of normal intramuscular adipose tissue accumulation. It might be

difficult to distinguish between minimal MSt and maximal accumulation of adipose cells in muscles showing a normal reduction in apparent number of myofibres.

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extramuscular denervation usually results in atrophy rather than MSt. MSt probably results from a combination of myofibre damage, motor denervation, autonomic re-innervation and positive caloric balance occurring as a result of intramuscular denervation in a growing animal. The normal intramuscular adipose tissue pattern is retained in areas of MSt, and fatty acid composition is similar to subcutaneous fat with a high amount of unsaturated fatty acids. In naturally occurring MSt, denervation alone would be unlikely to cause a major lesion because of the efficiency of collateral re-innervation by surviving neurons.

It is possible that if intramuscular denervation had occurred in conjunction with muscle rupture, MSt rather than fibrosis would be the result. It may be no coincidence that MSt is typically observed in heavily muscled meat animals in locations (loin and hind limb) that might be damaged by muscular exertion during MSt in one area of a muscle locomotion or mating. might predispose adjacent areas to trauma on subsequent exertion, thus accounting for the considerable tracts of MSt that may occur. The alternative hypothesis to self-inflicted muscle damage is that MSt is due to a defective development of vascular tissues. Although blood vessels with abnormally thick walls surrounded by connective tissues may be observed in naturally occurring MSt, this might also be related to muscle damage.

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With classical histological techniques, intermyofibrillar lipid droplets were distinguished from interstitial granules (mitochondria), and both were found to be more abundant in "dark" myofibres. Lipid staining droplets occur in bovine fetuses and in the atrophic muscles of steers on a submaintenance diet. The abnormal accumulation of lipid droplets may occur in myofibres either as a non-specific response to myofibre degeneration or through a defect in long chain fatty acid utilization. It is possible that lipid accumulation myopathy is an initial cause of MSt.

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sections Traverse muscle in myopathic conditions, polygonal cells resembling myofibres have a glassy appearance, are uniformly sudanophilic and are not exhibiting any reaction for beta-hydroxybutyric dehydrogenase, as are adjacent red myofibres. microscopy, myofibrillar disruption, lipid light infiltration and loss of birefringence can be observed within porcine Subsequent electron myofibres. microscopy shows that changes can be dissociation of groups of myofibrils, contraction of sarcomeres, loss of density in the A band and fragmentation of myofibrils. Lipid infiltration is confirmed, and it is also observed that the sarcolemma is detached and thickened and that mitochondria have wasted matrices and fragmented cristae.

Human lipid accumulation myopathies most often involve the red or type 1 myofibres is no coincidence that aerobic metabolism, the typical function of red myofibres, is deficient in SS-lineage pigs and that red myofibres are more easily damaged by ischaemia.

Different molecules, growth hormones, growth factors, lipids and other have been studied in

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association with the adipogenesis and myogenesis mechanisms. Among those factors, there is considered acidic and basic fibroblast growth factor (aFGF, FGF-2), transforming growth factor -beta and -alpha (TGF- α and TGF- α), adipocyte differentiating related protein (ADRP), epidermal growth factor (EGF), insulin like growth factor 1 and 2 (IGF-1 and IGF-2), IGF-1 receptor and IGF-2 receptor, platelet derived growth factor - alpha and -beta (PDGF- α and PDGF- β), leptin, and lipoprotein lipase (LPL).

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Epidermal growth factor (EGF) is 6-kDa molecular weight polypeptide found in high concentrations in the submaxillary glands and at lower circulation. EGF affects levels in the the proliferation and the maintenance of functional properties of various mammalian cells in vitro (13-14). Animal experiments involving either injection of EGF, injection of antibodies specific for EGF, or removal of the major source of EGF by sialoadenectomy, have shown that EGF played a physiological role on the maintenance of several tissue functions in vivo.

IGF-I and IGF-II are growth factors that have related amino acid sequence and structure, with each polypeptide having a molecular weight of approximately 7.5 kilodaltons (kDa). IGF-I mediates the major effects of growth hormone, and thus is the primary mediator of growth after birth. IGF-I has also been implicated in the actions of various other growth factors, since treatment of cells with such growth factors leads to increased production of IGF-I. In contrast, IGF-II is believed to have a major role in fetal growth. Both IGF-I and IGF-II have insulin-like activities (hence their names), and are mitogenic (stimulate cell

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division) and/or are trophic (promote recovery/survival) for cells in neural, muscular, reproductive, skeletal and other tissues.

Unlike most growth factors, IGFs are present in substantial quantity in the circulation, but only a very small fraction of this IGF is free in the circulation or in other body fluids. Most circulating IGF is bound to the IGF-binding protein IGFBP-3. IGF-I may be measured in blood serum to diagnose abnormal growth-related conditions, e.g., pituitary gigantism, acromegaly, dwarfism, various growth hormone deficiencies, and the like. Although IGF-I is produced in many tissues, most circulating IGF-I is believed to be synthesized in the liver.

Almost all IGF circulates in a non-covalently associated ternary complex composed of IGF-I or IGF-II, IGFBP-3, and a larger protein subunit termed the acid labile subunit (ALS). The IGF/IGFBP-3/ALS ternary complex is composed of equimolar amounts of each of the three components. ALS has no direct IGF binding activity and appears to bind only to the IGF/IGFBP-3 binary complex. The IGF/IGFBP-3/ALS ternary complex has a molecular weight of approximately 150 kDa. This ternary complex is thought to function in the circulation "as a reservoir and a buffer for IGF-I and IGF-II preventing rapid changes in the concentration of free IGF.

One other of these, the Insulin-Like Growth Factor-I Receptor (IGF-IR) is a member of the tyrosine kinase family of signal transducing molecules. The IGF-IR is activated by the ligands IGF-I, IGF-II and insulin at supra-physiological concentrations, and plays an important role in the development, growth, and

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survival of normal cells. Over-expression of the IGF-IR leads to the transformation of fibroblasts and conversely, IGF-IR null fibroblasts are refractory to transformation by a number of oncogenes. Fibroblasts from IGF-IR null mice have been used to demonstrate a requirement for the IGF-IR in transformation, and also to map domains in the receptor essential for the proliferative and transformation function of the IGF-IR. Specifically, the C-terminal region of the IGF-IR is required for the transformation function. Receptors, which are truncated at amino acid 1229 fail to transform fibroblasts derived from IGF-IR, null mice, but retain full proliferative activity.

PDGF is considered to be a principal growthregulatory molecule responsible for smooth muscle cell proliferation. For instance, PDGF, as measured by mRNA analysis as well as in situ staining using an antibody against PDGF, was found within macrophages of all stages of lesion development in both human and nonhuman primate atherosclerosis. PDGF was found in both nonfoam cells and lipid rich macrophage foam cells. These data are consistent with PDGF playing a critical role in the atherosclerosis disease process. In addition, analysis of advanced human lesions examined atherectomy catheter indicates that atherosclerotic and restenotic lesions contain high levels of PDGF as measured by in situ hybridization.

Human transforming growth factor-beta (TGF-beta) has been isolated from human blood platelets and placenta and purified to essential homogeneity using sequential gel filtration cation-exchange chromatography and high performance liquid chromatography. The purified protein has been

characterized as having a molecular weight of 25,000 daltons and composed of 2 sub-units of 12,500 daltons each held together by disulfide bonds. The molecular weight, sub-unit structure and amino acid composition of the purified protein differed from that of platelet derived growth factor.

TGF-beta has also been purified from platelets or conditioned media utilizing acid ethanol extraction, cation-exchange separation on the extract, and hydrophobic separations on the active fractions to obtain a homogenous preparation. The purified product is said to be useful in wound healing and tissue repair.

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TGF-beta has also been prepared utilizing recombinant DNA, wherein the cloned human gene coding for TGF-beta was inserted into eukaryotic cell lines for expression. The protein product was said to be useful in promoting anchorage-dependent or independent growth in cell culture.

The idea that FGF-2 antagonists may have useful medicinal applications is not new. FGF-2 is now known to play a key role in the development of smooth-muscle cell lesions following vascular injury. Overexpression of FGF-2 (and other members of the FGF family) is correlated with many malignant disorders (Takahashi et al. (1990) Proc. Natl. Acad. Sci. U.S.A. 87:5710). Neutralizing anti-FGF-2 antibodies have been found to suppress solid tumor growth in vivo by inhibiting tumor-linked angiogenesis (Hori et al. (1991) Cancer Res. 51:6180). Notable in this regard is the recent therapeutic examination of suramin, a polysulfated naphthalene derivative with known antiprotozoal activity, as an anti-tumor agent. Suramin is believed

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to inhibit the activity of FGF-2 through binding in the polyanion binding site and disrupting interaction of the growth factor with its receptor (Middaugh et al. (1992) Biochemistry 31:9016). In addition to having a number of undesirable side effects and substantial toxicity, suramin is known to interact with several other heparin-binding growth factors, which makes linking of its beneficial therapeutic effects to specific drug-protein interactions difficult. Antiangiogenic properties of certain heparin preparations have also been observed (Folkman et al. (1983) Science 221:719; Crum et al. (1985) Science 230:1375) and these effects are probably based at least in part on their ability to interfere with FGF-2 signaling. While the specific heparin fraction that contributes to FGF-2 binding is now partially elucidated, a typical heparin preparation is heterogeneous with respect to size, sulfation and iduronic acid content. degree of Additionally, heparin also affects many enzymes and growth factors. Basic FGF is thought to regulate myogenesis during muscle development and regeneration in vivo. The increase percentage of muscle fibers containing the donor gene produced by the addition of FGF-2 may seem surprising since FGF-2 was reported to inhibit differentiation of myoblasts in vitro. Basic FGF is, however, one of many growth factors, which are liberated following muscle damage. These factors, all together, certainly increase myoblast proliferation and ` eventually muscle repairs. It has been also observed that following two days incubation with FGF-2 of primary myoblast cultures, myoblast fusion occurred within a few days after removal of FGF-2. inhibition by FGF-2 on myoblast fusion is therefore not

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irreversible. Basic FGF is already at an increased level in mdx muscle, therefore it is not surprising that direct intramuscular injection did not increase the fusion of the donor myoblasts with the host fibers. In fact, FGF-2 injected directly in the muscle probably stimulates the proliferation of the host as well as the donor myoblasts and therefore does not favor the donor myoblasts. On the contrary, preliminary stimulation by FGF-2 of the donor myoblasts in culture may favor these myoblasts proliferate more and eventually. to participate more to muscle regeneration than the host myoblasts. Although FGF-2 stimulates the fibroblasts, a result, which could pose an inconvenience to primary myoblast cultures, the 48 hours incubation of myoblast primary culture with FGF-2, did not adversely affect the transplantation results. In fact, to the contrary, it improved them. If primary myoblast cultures were made fibroblast-free by sub-cloning, it would be envisageable to precondition the donor's myoblasts for a longer time, thereby increasing the number of cells to be transplanted from a relatively small biopsy.

In the capillary bed of the peripheral circulatory system, the enzyme lipoprotein lipase hydrolyzes and removes most of the triglycerides from the chylomicron. The lipoprotein that remains, now rich in cholesterol esters and potentially atherogenic, is called a chylomicron remnant. This postprandial lipoprotein is then removed from the circulation by the liver.

Other products or metabolic agents can be discussed, as such superoxide dismutase, carnitine, creatine, vitamin E, and lipids.

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The discovery of mutations to Cu, Zn superoxide dismutase in a subset of familial amyotrophic lateral sclerosis(ALS) cases has raised hopes for understanding the selective vulnerability of motor neurons as well as the pathogenesis of the remaining 98% of ALS cases not related to superoxide dismutase mutations.

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Neurofilaments give axons their structural integrity and define axonal diameter. Neurofilaments are composed of three subunits identified as light (NF-L), medium (NF-M) and heavy (NF-H) which assemble in a 6:2:1 ratio to form long macromolecular filaments. Consequently, NF-L is more abundant than the other two subunits in neurons. NF-L is capable of homologous assembly whereas NF-M and NF-H are not competent to assemble in the absence of NF-L. Each neurofilament subunit consists of conserved head and rod domains and a more variable acidic tail domain. The rod domains are principally composed of alpha helixes, which wrap around each other to form a superhelix of parallel coiled coils.

Amyotrophic lateral sclerosis is fatal neurodegenerative disease characterized by selective loss of motor neurons and accompanying loss of voluntary muscular function. ALS typically begins as weakness in one limb during middle adult life and progresses via contiguous groups of motor neurons to ultimately result in paralysis and death within 3-5 years. Ninety percent of ALS cases are sporadic with no identifiable genetic or environmental risk factors. A familial inheritance pattern has been observed in the remaining 10% of ALS cases and one-fifth of those result from dominant missense mutations to the antioxidant enzyme copper, zinc superoxide dismutase

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(Cu, Zn superoxide dismutase). Early histopathological changes in ALS include abnormal accumulations of neurofilaments and other cytoskeletal proteins in the cell soma as well as within proximal axonal swellings. The clinical course and histopathology of sporadic and familial forms of ALS are similar, providing hope that understanding superoxide dismutase-associated ALS was illuminate the pathogenesis of sporadic ALS.

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L-carnitine serves two major functions. best known for its role in facilitating entry of long-10 chain fatty acids into mitochondria for utilization in energy-generating processes. Long-chain fatty acids, as coenzyme A esters, are trans-esterified to Lcarnitine in a reaction catalyzed by carnitine 15 palmitoyltransferase I of the mitochondrial outer membrane. Long-chain acylcarnitine esters enter into mitochondria via a specific carrier, carnitineacylcarnitine translocase. On the matrix side of the inner mitochondrial membrane the long-chain fatty acid is transesterified to intramitochondrial coenzyme A, catalyzed by carnitine palmitoyltransferase Carnitine may exit the mitochondrion as such or as a short-chain acylcarnitine ester, via the translocase. This function of carnitine is obligatory: long-chain fatty acids cannot enter mitochondria independent of translocation as an ester of carnitine.

L-carnitine also facilitates removal from mitochondria of short-chain and medium-chain fatty acids that accumulate as a result of normal and abnormal metabolism. Short- and medium-chain acids, as acyl-CoA esters arising from $\beta\text{-oxidation}$ and other mitochondrial processes, are trans-esterified carnitine by the action of carnitine acetyltransferase.

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The acylcarnitine esters subsequently are transported out of mitochondria by the carnitine acylcarnitine translocase. This pathway provides a means to regenerate intramitochondrial free coenzyme A under conditions where short-chain acyl-CoA esters are produced at a rate faster than they can be utilized.

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Pharmacological administration of L-carnitine the mortality and metabolic consequences reduces associated with acute ammonium intoxication in mice. The mechanism associated with this effect may have two components: L-carnitine administration normalizes the redox state of the brain (perhaps by increasing availability of β -hydroxybutyrate to the brain), and it increases the rate of urea synthesis in the liver. At least part of the protective effect of L-carnitine is carnitine with associated flux through the acyltransferases, as inhibition of these enzymes by DLaminocarnitine, acetyl-DL-aminocarnitine, or palmitoyl-DL-animocarnitine enhances toxicity of acute ammonium Carnitine administration may have administration. significant benefit in patients with disorders of ammonia metabolism, including urea cycle defects, chronic valproic acid therapy, liver failure, organic acidemias, and Reye's syndrome.

It is known that propionyl-L-carnitine protects the ischemic heart from reperfusion injury, perhaps by scavenging free radicals or by preventing their formation by chelating iron necessary for generation of hydroxyl radicals. Long-chain acylcarnitine esters also participate in turnover and repair of erythrocyte membrane phospholipids, independent of ATP hydrolysis. It has been speculated that carnitine and its esters protect cells from oxidative damage, both by inhibiting

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free-radical propagation and by contributing to repair of oxidized membranes phospholipids. These processes may occur in many cell types, but may be particularly important in cardiac and other red muscle.

In poultry supplemented diet, it is not yet clear if the carnitine and its derivatives have an effect on feed intake, body and abdominal fat weight or on carcass or liver lipid levels.

Vitamin E acts to prevent the production of peroxide lipid as peroxide of an unsaturated fatty acid that is considered to be a material cause of the aging phenomenon. It has also a function of reinforcing blood vessels and activating the bloodstream, provides an anti-stress effect, and is a very important nutrient for human beings and other animals.

On the other hand, in stockbreeding, marine culturing or pet breeding, the problems of aging, reduced disease resistance, stress generation, decreased hatchability, deteriorated egg quality or meat quality, propagation disorder or mastitis, or reduction in the number of somatic cells in milk affect these animals, and a solution of these problems has hitherto been keenly demanded.

In the breeding of useful mammals including livestock animals such as cattle, pigs and horses, and pets such as dogs and cats, and experimental animals such as rats, mice and guinea pigs, reproduction is efficient because these animals are useful for human beings. As the breeding density increases, the 30 acceleration of aging, reduced disease resistance, stress generation, accelerated oxidation of meat foods, deteriorated meat quality such as the blackening of meat foods, and propagation disorder occur more often.

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Propagation disorder is caused by premature birth, reduction of conception ratio, ovulatory retardation, embryo death, a weakened estrous symptom or reduced production of progesterone.

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Poultry such as domestic fowl, quail and turkey under overcrowded breeding conditions suffer from reduced disease resistance, stress generation, deteriorated meat quality and propagation disorder, and additionally, reduced egg quality in the case of egg layers. In order to overcome these problems, various vitamins, including vitamin E and derivatives thereof, have been conventionally and minerals individually or in combination to the drinking water or feed and then fed to poultry.

Creatine occurs in muscle and nervous tissue (especially in the CNS), and in the form of its secondary metabolite, phosphocreatine, represents an energy reserve for muscle and brain. In the nervous and cardiac muscle tissue creatine appears to have a prophylactic and therapeutic effect in cases of ischemia resulting for instance from infarcts or preor perinatal conditions of oxygen deficit.

Creatine is not only an endogenous substance and a valuable food supplement but also has valuable therapeutic properties. It has been known for over a hundred years as a muscular substance and serves as a source of energy for the muscle. It was shown in a series of scientific studies that the intake of creatine can lead to an increase in muscular tissue and muscular performance.

There are also scientific findings that indicate that the pancreas releases more insulin under the influence of creatine. Insulin promotes the uptake of

glucose and amino acids by muscle cells and stimulates protein synthesis. Insulin also lowers the rate of protein catabolism.

The prophylactic, therapeutic or dietetic use of creatine in the most varied of application forms (oral, intravenous etc.) necessitates good bioavailability, which in turn means high solubility in water. This requirement is not sufficiently fulfilled in the case of creatine, which, as an amino-acid derivative, is present in the form of an internal salt.

None of the molecule mentioned above, as mature factor or as genetic marker, was considered as involved in the muscular steatosis metabolisms. None of the references disclosed above disclose or suggest the measurement of MSMFs to establish the health status regarding the steatosis, and their use for the treating or alleviating the symptoms of associated disorders. Further, none of the cited references disclose or suggest the administration MSMF alone or in combinations for treating or alleviating the symptoms of the muscular steatosis.

It would be highly desirable to be provided with a new method of modulating factors responsible of modulation of the steatosis status in human and animals. It is to this activity, and its applications in the modulation of steatosis through measurements of MSMF, selecting individuals regarding results of measurements, and administering MSMF to individuals if desired that the present invention be directed.

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SUMMARY OF THE INVENTION

One object of the present invention is to provide a method for prognosis or diagnosis of muscular

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steatosis based on the level of muscular steatosismodulating factor (MSMF) in a human or animal, comprising the steps of measuring level of at least one MSMF in a biological sample of a patient, and comparing the patient MSMF level with the MSMF level of a healthy human or animal, wherein a statistically significant difference indicate predisposition or occurrence of steatosis.

According to an object of the present invention, the method is addressed to animals selected from the group consisting of mammal, and avian, and most particularly, the animals selected from the group consisting of porcine, bovine, ovine, caprine, chicken, turkey, horse, goat, canine, and feline.

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Identifying differential expression of selected MSMF genes may perform the measurement of MSMF.

The MSMF may be selected from the group consisting of growth hormone, growth factor, cytokine, growth factor receptor, growth hormone receptor, cytokine receptor, and lipid.

The measured MSMF may also be measure of IGF1, IGF2, aFGF, FGF-2, ADRP, IGF1R, PDGF α , TGF β , TGF α , LPL, EGF, PDGF β , Leptin, superoxide dismutase, carnitine, creatine kinase, vitamin, or a combination thereof.

MSMF may be measured in a biological sample that may be derived from a sample of blood, serum, plasma, biopsy, fat, salivary, feces, or urine.

Also, measuring level of at least one peptide, a precursor, a metabolite, or a messenger RNA of MSMF performs the method according to the invention.

In accordance with another object, there is provided a method for the treatment of muscular steatosis in a human or animal patient, which comprises

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regulating MSMF level substantially equivalent to that of healthy patient by administrating an agonist, an antagonist of MSMF, or a combination thereof.

The treatment of steatosis may be performed by administration of an agonist of MSMF that is at least one MSMF.

The agonist may be a recombinant, a precursor, a non-mature, an analog, a purified, or a physiologically active fragment of at least one MSMF.

10 Also, the agonist of MSMF may be an abzyme.

In another embodiment of the invention, the treatment of steatosis may be performed by administration of antagonists of MSMF that are MSMFs.

Among embodiments of the present invention, the
antagonist of MSMF used to treat the steatosis may be
an abzyme.

According to the present invention, the antagonist may be selected from the group consisting of antibody, anti-MSMF messenger RNA, MSMF RNA ligand, MSMF-specific antisense primer, anti-MSMF receptor, and mutant MSMF.

Another particular embodiment of the present invention is that agonist, antagonist, or combination thereof may be administered by introducing at least one expression vector into the human or animal.

The expression vector may further be within at least one cell, and the cell is then introduced into a human or an animal to allow the *in vivo* synthesis of at least one agonist or antagonist of MSMFmay be administered systemically, orally, or intravenously, using an implant, or a slow delivery system.

According to the method of the invention, the muscular steatosis may be caused in an animal for

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increasing fat content in food, which comprises the step of administrating to the animal a sufficient amount of at least one agonist, antagonist of MSMF, or a combination thereof.

the invention is Another object of steatosis is caused by administration of agonist of MSMF that is at least one MSMF, or antagonist of MSMF that is at least one inhibitor of MSMF.

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The steatosis may be caused by administration of an agonist, or an antagonist selected respectively from the group consisting of recombinant, precursor, non mature, analog, purified, and a physiologically active fragment of at least one MSMF, or an inhibitor of recombinant, precursor, non mature, analog, purified, and a physiologically active fragment of at least one MSMF.

The antagonist according to the method of causing the steatosis may be selected from the group of an antibody, an anti-MSMF messenger RNA, a MSMF RNA ligand, a MSMF-specific antisense primer, an anti-MSMF receptor, a synthetic antisense, a natural antisense, and a mutant MSMF.

The messenger RNA or anti-MSMF messenger RNA may be complementary or corresponding to nucleic acid sequences selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305, or a fragment thereof.

Agonist of MSMF, antagonist of MSMF, combination thereof may be administered by introducing at least one expression vector into the human or animal, wherein the expression vector may be within at least one cell, and the cell is then introduced into a host human or animal.

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Another object of the method of causing the steatosis, is the administration of an agonist or antagonist systemically, orally, or intravenously, using an implant, or a slow delivery system.

In accordance with the present invention, there is provided a compound of the group of MSMF for treating or inducing muscular steatosis in a human or an animal patient.

The compound may be selected from the group 10 consisting of agonist, antagonist of MSMF, or a combination thereof.

In accordance with the present invention, there is provided a use of a compound of the group of MSMF in the manufacture of a medicament for treating or inducing muscular steatosis.

In accordance with the present invention, there is provided a pharmaceutical composition for use in treating or causing muscular steatosis comprising a therapeutically acceptable and effective amount of a compound of the group of MSMF in association with a pharmaceutically acceptable carrier.

For the purpose of the present invention the following terms are defined below.

The term "growth factor" as used herein refers
to any receptor ligand that causes a cell growth and/or
cell proliferation effect. Examples of growth factors
are well known in the art. Fibroblast growth factor
(FGF) is one example of a growth factor.

The term "recombinant product" as used herein refers to the product produced from a DNA sequence that comprises at least a portion of the MSMF. This product can be a peptide, a polypeptide, a protein, an enzyme, an antibody, an antibody fragment, a polypeptide that

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binds to a regulatory element (a term described hereafter), a structural protein, an RNA molecule, and/or a abzyme, for example. These products are well defined in the art.

By "expression vector" is meant any nucleic acid molecule or virus containing regulatory elements or reporter genes for the purpose of expression of a given gene in prokaryotic or eukaryotic cells or organisms. Such vectors can be introduced into a cell by means of molecular biological techniques. After introduction this cell, nucleic acid can exist into the extrachromosomally or become integrated into the host genome.

The term "abzyme" as used herein means antibody directed enzyme prodrug. Abzymes are defined as antibodies directed against appropriate transition state analogues that can catalyse a variety of chemical transformations and metabolic reactions. Furthermore, murine antibodies can be "humanized" using existing technologies to reduce their immunogenicity patients. Thus a humanized catalytic antibody (abzyme) could be prepared which replaces an enzyme and thus leads to a treatment system that combines specificity and low immunogenicity.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials described herein can be used to practice the 30 present invention, other similar or equivalent methods and material known to one skilled in the art can also used. All publications, patent applications, patents, and other references mentioned herein are

incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, was control. The materials, methods, and examples described herein are illustrative only and not intended to be limiting.

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BRIEF DESCRIPTION OF THE DRAWING

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Fig. 1 illustrates level expression (RT-PCR) of studied genes for muscular fat as steatosis markers in healthy pigs and pigs having high degree of steatosis.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a new method of modulating levels of muscular steatosis-modulating factors (MSMF), measuring levels of human and animal with naturally occurring or administered MSMF. As shown in examples provided below, measurement of steatosis based on measured levels of MSMF could be made by comparison to levels measured in a steatosis-free control group or background level measured in a particular subject. The measurement can be confirm by correlation of the assay results with other aforementioned methods of disease known to those skilled in the arts, such as photonic microscopy. Among MSMFs of the present invention, there is considered acidic and basic fibroblast growth factor (aFGF, FGF-2), transforming growth factor -beta and -alpha (TGF- β and TGF- α), adipocyte differentiating related protein (ADRP), epidermal growth factor (EGF), insulin like growth factor 1 and 2 (IGF-1 and IGF-2), IGF-1 receptor and IGF-2 receptor, platelet derived growth factor -alpha and -beta (PDGF- α and PDGF- β), leptin, and lipoprotein lipase (LPL). Lipids that can

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be considered in establishing the steatosis status for monitoring MSMF of an individual are myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), palmitic acid (C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), margaroleic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linoleinic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4), beneic acid (C22:0), erucic acid (C22:1), docosandienoic acid (C22:2), docosahexaenoic acid (C22:6), and lignoceric acid (C24:0).

In another embodiment of the invention, there is provided a method of detecting and quantifying MSMF in biological samples using an antibody specific for MSMF and, where appropriate, a detectable-labeled antigen (MSMF). The invention is to provide methods for diagnosis of diseases that are correlated to the loss and/or synthesis of muscular tissue as indicated by levels of MSMF or lipids detected in a biological sample. A method of identifying differential expression of selected genes is used to diagnosing the muscular steatosis in human and animals.

In another embodiment of the present invention, there is provided measure levels of FGF-2, IGF1R and LPL alone or in combinations as genetic markers in determining sings of muscular steatosis in a human or an animal.

In another embodiment of the present invention, there is provided a method for determining the steatosis status by using reverse transcription and polymerase chain reaction to amplify small amounts of

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MSMF mRNA. DNA-DNA hybridization can then be used to confirm the specificity of the amplified product as being MSMF.

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This technique provides a method for measuring the quantities of MSMF. The ability to demonstrate the quantities of MSMF by RT-PCR and then confirm the specificity of the amplification by DNA hybridization has significant implications in clarifying MSMF role in muscular steatosis. In practice it is rendered possible 10 a direct testing of biological samples for the presence of MSMF that may be conducted.

The invention further provides screening methods to identify concentration of molecules that can be involved in modulating steatosis. In one aspect, such screening methods comprise competitive binding assays wherein the ability of a putative modulating molecule to bind to MSMF is measured in the presence of a suitably labeled C-terminal peptide.

In one embodiment of the invention, MSMF are measured to selected animals having specific characteristics regarding targeted MSMF. Those animals selected to be exempted of any sing of steatosis may be considered as genetically qualified for establishing For example, farm production of porcine, lineages. bovine, chicken, turkey, ovine and caprine should profit of genetically selected founders establishment of healthy herds through the present invention.

In another embodiment, the invention is directed the selection of stably genetically selected individuals having naturally different status of muscular steatosis, to serve as founder animals for the establishment of specific herds having these

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properties. It is well recognized that lipids and ratios of muscular lipids can influence the texture and taste of the meat. In some cases, higher level of muscular steatosis may be suitable to have animal with more fatty muscles.

Alternatively, agonists of positively inducing MSMF, or MSMF itself can be administered to an animal to induce steatosis for the same aim mentioned above. Agonists of MSMF, for example, can be a MSMF itself or combinations of MSMF, or abzymes that mimic binding sites of MSMF to their respective cell receptors, or that mimic enzymatic activity of the MSMF. Antagonists of MSMF, can be administered to reestablish a healthy state of an individual affected by the muscular steatosis.

Yet additional embodiments of the invention comprise the use of MSMF and lipid compositions of the invention as screening markers for molecules which modulate or are involved in the establishment of muscular steatosis. Such embodiments include, but are not limited to, assays which measure the ability of a putative MSMF to compete with other peptides and proteins (including, but not limited to, other peptide sequences of the MSMF itself), which are identified to act specifically to the receptor compositions of the invention, in order to modulate the steatotic state of an individual.

The immunoassay procedure used is preferably quantitative so that levels of MSMF in a patient with disease may be distinguished from normal levels which may be present in healthy individual and/or background levels measured in the patient. Competitive and sandwich assays on a solid phase using detectable

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labels (direct or indirect) are, therefore, preferred. The label provided a detectable signal indicative of binding of antibody to the MSMF antigen. The antibody or antigen may be labeled with any label known in the detectable signal, art provide a fluorescent molecules, radioisotopes, enzymes, chemiluminescent molecules, bioluminescent molecules and colloidal gold. Of the known assay procedures, radioimmunoassay (RIA) is most preferred for sensitivity. A radioisotope had, therefore, is the preferred label.

It has been appreciated by those skilled in the art that, although not necessarily as sensitive as an procedures using labels other assay than radioisotopes have certain advantages and may, therefore, be employed as alternatives to the preferred RIA format. For example, an enzyme-linked immunosorbent assay (ELISA) may be readily automated using an ELISA micrometer plate reader and reagents who are readily available in many research and clinical laboratories. Fluorescent, chemiluminescent and bioluminescent labels have the advantage of being visually detectable, though they are not as useful as radioisotopes to quantify the amount of antigen bound by antibody in the assay.

Molecules identified by means of the screening assays of the invention has been candidates as useful therapeutic products for the *in vivo*, *ex vivo* or *in vitro* treatment of target tissues alone or in combination with suitable carriers and excipients. Such compositions and their use comprise additional embodiments of the invention.

In yet another embodiment of the invention, there is provided expression vectors containing genetic

sequences, hosts transformed with such expression vectors, and methods for producing the recombinant MSMF compositions of the invention.

The present invention is further directed to methods for inducing or suppressing apoptosis in the cells and/or tissues of individuals suffering from characterized by inappropriate cell disorders proliferation or survival, or by inappropriate cell respectively. Disorders characterized death, inappropriate cell proliferation and/or survival include, for example, inflammatory conditions, cancer, including lymphomas, genotypic tumors, etc. Disorders characterized by inappropriate cell death include, for example, autoimmune diseases, acquired immunodeficiency disease (AIDS), cell death due to radiation therapy or chemotherapy, acute hypoxic injury, etc.

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In another embodiment of the present invention, there is provided a method for identification of the hormones and other factors, the steatosis-modulating factors, controlling the balance between muscular and adipocyte proliferation and differentiation, that is very important for modulating normal adipose and muscular tissue development and for designing approaches for screening individuals having normal and abnormal states of adipose tissue development, such as obesity for example.

In yet another embodiment of the present invention, there is provided a method of treating an individual with MSMF in an individual that need such treatment, comprising the step of administering to the individual a pharmacologically effective dose of one MSMF aforementioned or combinations thereof.

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The present invention was more readily understood by referring to the following examples, which are given to illustrate the invention rather than to limit its scope.

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EXAMPLE I

MUSCLE-FAT IMBALANCEMENT IN GROWING PIGS

In swine, specific ham muscles such as semitendineous biceps femoris and semi-membranous are sometimes abnormally infiltrated with fat, leading to a severe muscle degeneration. We suspected different genetic factors to be implicated in the development of this muscular-fat imbalance.

A total of 113 among 676 pigs were selected in a local farm. Healthy and steatotic animals were directly selected at the farm by using ALOKA apparatus performing bi-directional ultrasonic reading. After slaughter, 80 pigs were retained following a visual quotation of the left semi-tendineous muscle and according to a design with muscular fat infiltration (0 or severe).

Methods of analysis

Vitamin E: The concentrations of plasmatic and hepatic Vitamin E were determined through an home made adapted method described by Bieri et al (Bieri, J.G. et al.,Am. J. Clin. Nutri.(1979) vol. 32; 2143-2149) on HPLC (High Pressure Liquid Chromatography). Results are presented in Table 1.

L-carnitine: Using a modified approaches (radio-isotopical) developed by McGarry and Foster ((1976) J. Lipid Res. 17:277-281), concentrations of L-carnitine contained in semi-tendineous muscles and plasma were determined. Results are shown in Table 1.

Creatine kinase: The analysis of quantities of creatine kinase in plasma has been performed with a commercial (Sigma Diagnostics #C2527, St-Louis, MO) enzymatic kit allowing to measure variations of NADH at optical density of 340 nm, that is a direct indication of the creatine kinase activity. Results are presented in Table 1.

Selenium: Quantities of selenium in blood was directly measured by the assessment of the activity of glutathion peroxidase. The level of activity is determined by measuring oxidative rates of NADPH by spectrophotometry at 340 nm of optical density. Results are presented in Table 1.

Superoxide dismutase: The activity of superoxide-dismutase in muscles was performed with a commercial kit(Calbiochem, #574600, San Diego, CA) allowing to measure variation in levels of oxidation of a chromophore agent by optical density at 525nm.

Results are shown in Table 1.

TABLE 1
SUMMARY OF STATISTICS OF BLOOD AND TISSUE ANALYSIS

		Р						
Variables	M	F	Affected	Normal	SEM	Sex	Steatosis	S/ST
CARNITINE MUSCLE (nmoles/mg protein)	22.01	21.32	21.52	21.81	0.644	0.451	0.753	0.574
CARNITINE PLASMA (umoles /liter)	4.67	4.42	4.88	4.21	0.213	0.408	0.029	0.656
CREATINE KINASE PLASMA (Unit/liter)	382.0	418.4	360.9	439.6	49.625	0.613	0.276	0.536
SELENIUM PLASMA (nmoles/mg protein)	17.002	17.724	17.527	17.199	0.769	0.508	0.764	0.558
SELENIUM BLOOD (umoles/min./gra m Hb)	231.32 3	222.35 6	228.020	225.658	9.019	0.484	0.854	0.456
SUPEROXIDE DISMUTASE MUSCLE (Unit/mg protein)	18.66	19.42	18.41	19.68	0.284	0.062	0.002	0.894
VITAMIN E LIVER (ug/gram liver)	20.886	20.243	22.691	18.438	0.859	0.600	0.001	0.279
VITAMINE E PLASMA (ug /ml)	1.188	1.371	1.268	1.291	0.064	0.050		0.957

⁵ Legend: M, males; F, females; S/ST, Sex*Steatosis; SEM, standard deviation/□n; (n = 40)P, probability; significant when < 0.05 (shaded)</p>

Patterns of muscular and sub-cutaneous fatty
10 acids were determined on gas phase chromatography.
Results are shown in Tables 2, 3 and 4.

TABLE 2

BACKFAT AND MUSCLE TISSUE FATTY ACIDS (%) IN NORMAL AND AFFECTED PIGS

•	SEX		STE	ATOSIS	Р				
Tissue Fat	M	F	Affected	Normal	SEM	Sex	Steatosis	S/ST	
FAT Mono	48.66		49.62	49.21	0.485	0.040	.7	0.018	
FAT Poly	17.14		16.34	17.57	0.492	0.605	0.092	0.971	
FAT SATURED	34.20		34.04	33.22	0.526	0.141	0.283	0.029年7月	
MUSCLE MONO	47.58		48.51	46.29	1.136	0.819	0.182	0.034	
MUSCLE POLY	14.39		11.92	18.33	0.727	0.170	0.001	0.105	
MUSCLE SATURED	38.01		39.56	35.35	0.956	0.422	0.005	0.182	

Legend: M, males; F, females; S/ST, Sex*Steatosis; SEM, standard deviation/ \sqrt{n} ; (n = 12) P, probability; significant when < 0.05 (shaded).

TABLE 3

BACKFAT FATTY ACID COMPOSITION (%) IN NORMAL AND
AFFECTED PIGS

		SEX			osis	Р				
FATTY ACIDS	TISSUE	М	F	Affected	Normal	SEM ·	Sex	Steatosis	S/ST	
C14:0	FAT	1.54	1.34	1.34	1.54	0.061	i⇒ 0:025	##10.031 ^{***}	0.001	
C14:1	FAT	0.08	0.06	0.05	0.10	0.015	0.341	0.041	0.296	
C15:0	FAT	0.15	0.11	0.10	0.16	0.022	0.172	0.060	0.180	
C15:1	FAT	0.08	0.07	0.05	0.09	0.010	0.368	B-0.024	0.252	
C16:0	FAT	22.09	20.80	21.28	21.60	0.448	0.056	0.618	0:006	
C16:1	FAT	2.89	2.77	2.57	3.09	0.087	0.324	J. 10.001	0.007	
C17:0	FAT	0.42	0.37	0.38	0.41	0.022	0.068	0.266	0.108	
C17:1	FAT	0.36	0.36	0.34	0.39	0.020	0.863	0.090	0.807	
C18:0	FAT	9.37	9.88	,10.41	8.83	0.194	0.079	0.001	0.020	
C18:1	FAT	42.87	44.36	43.46	43.77	0.510	0.053	0.669	0.136	
C18:2	FAT	14.47	13.97	13.72	14.73	0.444	0.434	0.124	0.985	
C18:3	FAT	1.29	1.29	1.21	1.36	0.051	0.972	0.045	0.567	
C20:0	FAT	0.22	0.24	0.24	0.22	0.011	0.239	0.295	0.070	
C20:1	FAT	2.23	2.39	3.05	1.57	0.585	0.848	0.089	0.231	
C20:2	FAT	0.61	0.70	0.67	0.65	0.028	0.039	0.644	0.053	
C20:3	FAT	0.17	0.16	0.15	0.18	0.012	0.650	0.128	0.781	
C20:4	FAT	0.26	0.32	0.29	0.29	0.022	0.058	0.792	0.096	
C22:0	FAT	0.15	0.16	0.13	0.17	0.039	0.830	0.453	0.306	
C22:1	FAT	0.14	0.17	0.10	0.21	0.028	0.461	0.015	0.506	
C22:2	FAT	0.16	0.17	0.14	0.20	0.020	0.849	0.044	0.450	
C22:6	FAT	0.17	0.16	0.17	0.17	0.026	0.795	0.925	0.026	
C24:0	FAT	0.24	0.17	0.15	0.27	0.033	0.097	0.023	0.494	

Legend:M, males; F, females; S/ST, Sex*Steatosis; SEM, standard deviation/ \sqrt{n} ; (n = 12)P, probability; significant when < 0.05 (shaded).

TABLE 4

MUSCLE FATTY ACID COMPOSITION (%) IN NORMAL AND AFFECTED PIGS

	>	F		rosis	STEAT	X	SE		
S/ST	Steatosis	Sex	SEM	Normal	Affected	F	М	TISSUE	FATTY ACID
0.216	₩.0.003 _€	" 0.028 ₄	0.118	1.28	1.84	1.36	1.76	MUSCLE	C14:0
0.101	0,025	0.583	0.026	0.18	0.09	0.13	0.15	MUSCLE	C14:1
0.186	0.731	0.158	0.052	0.19	0.17	0.13	0.23	MUSCLE	C15:0
0.026	0.001	0.390	0.074	0.89	0.27	0.63	0.53	MUSCLE	C15:1
0.197	701001	0.185	0.764	21.44	25.42	22.69	24.17	MUSCLE	C16:0
0.736	0.095	0.053	0.128	3.11	3.43	3.08	3.45	MUSCLE	C16:1
0.081	0.014	0.576	0.021	0.36	0.28	0.31	0.33	MUSCLE	C17:0
0.195	0.266	0.839	0.015	0.29	0.27	0.28	0.28	MUSCLE	C17:1
0.634	0.348	0.077	0.330	10.81	11.26	11.47	10.60	MUSCLE	C18:0
0.065	0.114	0.995	1.217	40.02	42.87	41.44	41.45	MUSCLE	C18:1
0.340	0.001	0.116	0.525	13.45	9.31	11.99	10.77	MUSCLE	C18:2
0.675	0:0441	0.775	0.046	0.96	0.82	0.90	0.88	MUSCLE	C18:3
0.357	0.206	0.693	0.042	0.34	0.26	0.31	0.29	MUSCLE	C20:0
0.171	0.845	0.909	0.387	1.53	1.42	1.51	1.44	MUSCLE	C20:1
0.044		0.915	0.037	0.59	0.51	0.55	0.54	MUSCLE	C20:2
0.050	0.004	0.930	0.046	0.43	0.22	0.32	0.33	MUSCLE	C20:3
0.046	0.001	0.201	0.203	2.26	0.60	1.62	1.24	MUSCLE	C20:4
0.045	0.001	0.931	0.034	. 0.31	0.12	0.22	0.21	MUSCLE	C22:0
0.290	0.161	0.078	0.045	0.26	0.17	0.15	0.27	MUSCLE	C22:1
0.360	0.002	0.445	0.043	0.41	0.19	0.28	0.32	MUSCLE	C22:2
0.066	0.621	0.138	0.049	0.25	0.28	0.21	0.32	MUSCLE	C22:6
0.236	0.001	0.953	0.074	0.61	0.21	0.41	0.41	MUSCLE	C24:0
((0.002	0.445	0.043 0.049	0.41	0.19	0.28	0.32	MUSCLE	C22:2 C22:6

Legend: MUSCLE, affected part of the muscle; M, males; F, females; S/ST, Sex*Steatosis; SEM, standard deviation/ \sqrt{n} ; (n = 12) P, probability; significant when < 0.05 (shaded).

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PCR amplification of messenger RNA (RT-PCR):

Separation of intramuscular fat and muscle fibers: In order to amplify the transcripts that correspond only to intramuscular fat or to muscle fibers, pieces of semi-tendineous were taken from the freezer and immediately placed under a binocular. Separation of intramuscular fat from muscle fibers was performed manually using a thin needle. Samples of intramuscular fat and muscle fibers were immediately transferred to tubes filled with 2 ml Trizol™ reagent (Gibco-BRL, Bethesda, MD). These tubes were kept at -80°C until needed.

RNA extraction: RNA was extracted in Trizol™ reagent according to the manufacturer's instructions. The extracted RNA was dissolved in water and quantified spectrophotometrically at 260 nm. RNA aliquots were electrophoresed in a 1% agarose gel to verify their integrity.

Quantitative RT-PCR: For all samples, 5µg of RNA was treated with 3 units of Dnase I (Amplification grade #8068-015, Gibco-BRL, Bethesda, MD) to remove contaminating genomic DNA. First strand cDNA was synthesized from 5 μ q of total RNA from either intramuscular fat or muscle fibers, usina SuperScriptTM II preamplification system for first strand cDNA synthesis (Gibco BRL, Burlington, ON) and .500 ng of oligo (dT) 12-18 primer in a total reaction volume of 50 μ l. An aliquot of 2 μ l of the reverse product subjected transcriptase was PCR amplification.

The following RT-PCR were performed for intramuscular fat: ADRP, EGF, IGF1, IGF2 IGF1R, IGF2R, PDGF α , PDGF β , TGF β , aFGF, FGF-2, TGF α , leptin, LPL and

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MEF2 as a control. In muscle fibers the following RT-PCR were performed: EGF, IGF1, IGF2 IGF1R, IGF2R, PDGF α , PDGF β , TGF β , aFGF, FGF-2, TGF α , LPL and leptin as a control.

For each gene, a 100 μ l PCR reaction contained 5 either 15 pmol or 30 pmol of upstream and downstream primers (see Table 5), 200 μM dNTPs, 1.5 mM MgCl₂ and 2.5 units of Tag™ polymerase in 1X Tag™ polymerase buffer (Amersham Pharmacia Biotech, Baie d'Urfée, QC). Each gene's PCR profile was performed using a 10 Programmable Thermal Controller PTC-100TM (MJ Research Inc., Watertown, MA). The PCR amplifications consisted of an initial denaturation step at 94°C for 2 min, followed by variable cycle numbers of denaturation at 94°C for 1 min (see Table 5), annealing at different 15 temperature for 1 min (see Table5), extension at 72°C for 1 min and a final extension at 72°C for 5 min. Pig glyceraldehyde-3-phosphate dehydrogenase (GAPDH) also used as an internal control of amplification. For GAPDH PCR amplification, the 100 μ l PCR reaction 20 contained 30 pmol of upstream and 30 pmol of downstream primers (Table 5), 200 μl dNTPs, 1.5 mM MgCl₂ and 2.5 units of Tag™ polymerase in 1X polymerase buffer. The GAPDH PCR profile consisted of an initial denaturation step at 94°C for 2 min, followed by 20 cycles of denaturation at 94°C for 1 min, annealing at 68°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 5 min.

TABLE 5
PCR Conditions

							Concen-	Hybrida-
Sondes	Primer	Primer Sequence	Position	size	Genebank	cycle	tration	tion Tempera-
	Site	(5'-3')	(pb)	fragments	no	numbers		ture
IGF 1	Forward Reverse	5' - GCA CAT CAC ATC CTC TTC GCA TC - 3' 5' - TGT ACT TCC TTC TGA GCC TTG GG - 3'	15-37 331-353	338	PIGGFIIA	25	30	70 C
IGF 2	Forward	5' - GGT GGA CAC CCT CCA GTT TGT C - 3' 5' - GTG ACG CTT GGC CTC TCT GAC -	481-502	354	SSIGF2	24	15	70 C
	Reverse	3'	815-835		-			
IGF1R	Forward	5' - CGC ATG TGC TGG CAG TAC AAC C - 3' 5' - TGC GCG TAA GGC TGT CTC TCG -	19-40	307	SSU15445	25	30	70 C
	Reverse	3'	306-326					
IGF2R	Forward Reverse	5' - GGC CAA GTC CAA CTG CCG CTA C - 3' 5' - ACT CAT CCG CTG GAA GCC CG - 3'	1-22 363-382	381	SSU58650	25	30	70 C
aFGF	Forward Reverse	5' - TGG CTG AAG GCG AAA TCA CAA CC - 3' 5' - TGA GTC CGA GGA CCG CGT TTG - 3'	17-39 411-431	414	SSAFGFRNA	25	30	70 C
bFGF	Forward Reverse	5' - ACG GAG GCT TCT TCC TGC GC - 3' 5' - CGT TCG TTT CAG TGC CAC GTA CC - 3'	138-157 399-421	283	SSBFGF	24	15	70 C
EGF	Forward Reverse	5' - ATC TCTAGA GCG CAG CTC CCA CCA TTT CAA GTC -	2607-2630 3482-3505		HSEGFRER	25	15	65 C

Sondes	Primer Site	Primer Sequence	Position (pb)	size fragments	Genebank no	cycle numbers	Concen- tration oligo (pmoles)	Hybrida- tion Tempera- ture
TGFa	Forward Reverse	5' - CTT GTT GGC CGT GTG CCA GGC - 3' 5' - AGC GGT CCT TCC CTT CAG GAG GG - 3'	54-74 443-465	411	SSTGFA	27	15	70 C
TGFb	Forward Reverse	5' - AAG CGG AAG CGC ATC GAG G - 3' 5' -GCG GCC CAC GTA GTA CAC G - 3'		995	GGTGFB1	25	30	70 C
PDGFa	Forward Reverse	5' - CCC GCG AGG TGA TCG AGA G - 3' 5' - GGC TTC TTC CTG ACG TAT TCC AC - 3'	464-482 850-872	408	HSPDGFAR	24	15	70 C
PDGFb	Forward Reverse	5' - GCG TCA CCG TGG CCT TCT TAA	1013-1036 1474-1496		HSPDGFB	24	15	70 C
ADRP	Forward Reverse	5' - ATC AAGCTT AAC AGA GCG TGG TGA TGA GAG TGG C - 3' 5' - ATC TCTAGA CCT ACC AGC CAG TTG AGA GGC G - 3'	27-50	1173	MMADRPCO D	27	15	70 C
LEPTIN (ob)	Forward Reverse	5' - GTC GAT TCC TGT GGC TTT GGC CC- 3' 5' - CTC CGT GGA GTA GAG GGA GGC TTC C - 3'	74-96 459-483	409	AF026976	24	15	70 C
LEPTIN (ob)	Forward Reverse	5' - GTC GAT TCC TGT GGC TTT GGC CC- 3' 5' - CTC CGT GGA GTA GAG GGA GGC TTC C - 3'	74-96 459-483	409	AF026976	35	15	70 C
MEF2 .	Forward Reverse	5' - AGA GCT GCT CAG ACT GTC CAC	1262-1284 1774-1797		HSMEF2	35	30	70 C

Sondes	Primer Site	Primer Sequence	Position (pb)	size fragments	Genebank no	cycle numbers	Concen- tration oligo (pmoles)	tion Tempera-
GAPDH	Forward Reverse	5' - CTG GCA AAG TGG ACA TTG TCG CC - 3' 5' - CTT GGC AGC GCC GGT AGA AGC - 3'	28-50	571	SSU48832	20	30	68 C
Lipoproteine	Forward	5' - GAG GGA ACC GGA TTC CAA CG - 3' 5' - AGG GCA TCT	475-494	709	SSLPLRNA	24	30	65 C
Lipase (LPL)	Reverse	GAG CAC GAG TC -	1165-1184					

The amplified PCR fragments were electrophoresed on a 2.5% agarose gel and stained with ethidium bromide. Pictures of the resulting gels were taken on 5 Polaroid film # 55. Films were then scanned using a densitometer (BIO-RAD™ Imaging Densitometer Model GS-670 Bio-Rad Laboratories Led., Mississauga, ON). The relative optical density of the transcripts expressed in arbitrary optical units. A ratio of the optical density of each transcript, standardized using the GAPDH transcript, was calculated before statistical analyses were performed to correct for possible differences in gel loading. The results are shown in Tables 6, 7, 8 and in Figure 1 representing the RT-PCR analysis of FGF-2 gene expression in muscle fibers of healthy (normal) and steatotic (affected) pigs, and where is amplified mRNA specific to the genes GAPDH (fragment of 571 bp) as control, and FGF-2 (fragment of 282 bp) as differential MSMF marker.

TABLE 6

EXPRESSION LEVELS (RT-PCR) OF DIFFERENT CANDIDATE
GENES IN INTRAMUSCULAR FAT OF NORMAL AND AFFECTED
PIGS

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	SE	ΣX	STEA	rosis		•	Р	
GENE	М	F	Affected	Normal	SEM	Sex	Steatosis	S/ST
ADRP	44.86	58.75	47.09	56.52	6.160	0.127	0.292	0.340
EGF	67.24	56.87	47.61	76.50	6.107	0.244	0.003	0.059
IGF1	55.10	69.78	65.19	59.69	4.686	0.039	1	0.518
IGF1R	59.24	64.79	71.78	52.24	3.354	0.256	0.001	0.830
IGF2	45.70	51.78	34.09	63.38	6.826	0.536	0.007	0.931
IGF2R	76.01	65.97	75.38	66.60	6.644	0.298	0.361	0.258
PDGFα	76.44	72.06	74.01	74.48	4.689	0.516	0.944	0.697
PDGF <i>β</i>	65.28	53.92	56.82	62.38	7.238	0.280	0.593	0.163
TGFβ	58.62	68.10	55.30	71.42	7.473	0.381	0.143	0.945
aFGF	69.74	59.74	56.22	73.26	5.128	0.183	0.029	0.198
FGF-2	37.79	51.46	53.38	35.87	3.198	d 0.007	0.001	0.001
TGFa	45.07	63.72	66.52	42.27	3.350	0.001	0.001	0.010
Leptin	53.50	64.15	68.81	48.83	3.231	0.030	0.001	0.040
LPL	76.23	60.19	65.92	70.49	5.389	0.048	0.556	0.281

Legend: M, males; F, females; S/ST, Sex*Steatosis; SEM, standard deviation/ \sqrt{n} ; P, probability; significant when < 0.05 (shaded). Values in this table correspond to relative optical density that were adjusted with respect to GAPDH transcript. For each gene, the highest expression value was considered 100% and transcripts of all pigs were adjusted relative to this pig.

TABLE 7

EXPRESSION LEVELS (RT-PCR) OF DIFFERENT CANDIDATE
GENES IN MUSCLE FIBERS OF NORMAL AND AFFECTED PIGS

	SI	€X	STEAT	OSIS			Р	
GENE	М	F	Affected	Normal	SEM	Sex	Steatosis	S/ST
FGF-2	56.32	50.21	36.72	69.81	5.167	0.413	4 0 001	0.960
EGF	64.94	65.38	69.84	60.49	6.317	0.962	0.308	0.171
IGF1	66.76	61.17	64.91	63.02	3.371	0.254	0.695	0.278
IGF1R	85.96	78.93	80.29	84.60	2.259	a 0.040	0.192	0.564
IGF2	72.96	49.09	55.68	66.38	6.215	0.013	0.238	0.720
IGF2R	75.98	57.28	73.43	59.83	5.418	0.024		0.706
PDGFa	68.76	51.01	66.70	53.07	3.959	.0.005	0.024	0.254
PDGF <i>β</i>	69.38	63.33	57.01	75.70	3.983	0.295	0.003	0.230
TGFa	49.81	48.36	47.25	50.92	5.900	0.864	0.665	0.491
aFGF	67.98	57.86	64.64	61.21	8.418	0.406	0.776	0.809
TGFβ	54.31	51.18	46.97	58.52	4.912	0.657	0.112	0.009
LPL _.	64.66	66.60	60.68	70.59	3.155	0.669	0.038	0.090

Legend: M, males; F, females; S/ST, Sex*Steatosis; SEM, standard deviation/ \sqrt{n} ; P, probability; significant when < 0.05 (shaded). Values in this table correspond to relative optical density that were adjusted with respect to GAPDH transcript. For each gene, the highest expression value was considered 100% and transcripts of all pigs were adjusted relative to this pig.

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TABLE 8

INTRAMUSCULAR LEVELS OF FGF-2, TGFA AND LEPTIN.

	normal females	normal males	affected females	affected males
		INTRAMUSCULAR	FAT	
FGF-2	34.20	37.54	68.71	38.05
TGFα	44.86	39.68	82.59	50,46
Leptin	49.13	48.54	79.17	58.46
		MUSCLE FIBER	S	
TGFβ	67.06	49.97	35.29	58.65

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Values in this table correspond to relative optical density that were adjusted with respect to GAPDH transcript. For each gene, the highest expression value was considered 100% and transcripts of all pigs were adjusted relative to this pig.

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EXAMPLE II

IDENTIFICATION OF MOLECULAR MARKERS

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Experimental method

Animal selection and sampling: 48 castrated commercial pigs from a same producer were used. These pigs were allocated according to a two-by-two factorial design in complete blocks with, as principal effects, the level of steatosis (24 pigs with steatosis levels 3 4; 24 normal pigs) and the adiposity level (24 fat pigs with $P_2 \geq 22$ mm between third and fourth ribs; 24 lean pigs with $P_2 \leq 19$ mm). The animals were selected at the slaughterhouse the morning of the day of slaughter using an ultrasound machine. For each selected pig, blood was drawn just prior to their slaughter. At slaughter, the entire left semi-tendinosus muscle was

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taken, the fat trimmed off and then cut transversally in order to evaluate the steatosis level. Wrongly identified pigs were immediately replaced by new pigs on the same day of slaughter. The two hams, the loin, the flank, the liver and a sample of backfat were taken for further biochemical and genetic analysis.

Validation of results obtained: This part of the example has allowed us to confirm the results obtained in the preceding example. More precisely, we 10 have performed the following analyses:

Measure of vitamin E: Vitamin E in the liver was performed by HPLC (high performance liquid chromatography) according to the protocol of Bieri et al. (1979, Am. J. Clin. Nutri. 32:2143-2149). First, the lipids were extracted using organic solvents (hexane or heptane) and the analysis of the tocopherols was done on a C18 column (inverse phase) which permits a fine separation of the different tocopherols.

Total carnitine levels: It was determined in plasma and in muscle, according to the radio-isotopic method developed by McGarry and Foster (1976, J. Lipid Res., 17:277-281).

All carcass and meat quality analyses, including pH at 45 minutes and ultimate pH was performed on all three studied muscles (semi-tendinosus, semi-membranosus and biceps femoris), and the loin and flank; allocation of the visual steatosis and marbling levels; determination of the percentage of dry matter of the loin, the flank and the three ham muscles; the percentage of lipids in the loin, the flank and the semi-tendinosus; measures of the backfat and muscle thickness as well as the muscle surface at the site of carcass classification (between the 3rd and 4th last

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ribs); water retention by the loin, the flank and the three ham muscles; the levels of glucose in the lost water; total protein content. The incidence of PSE meat was evaluated by measuring the color on the ventral side of the longissimus dorsi in the middle of the loin as well as on the three ham muscles studied. Digital images were taken of the transversal cuts of the studied muscles.

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Measure of sub-cutaneous and intra-muscular 10 fatty acids by gas chromatography: The intra-muscular lipids (semi-tendinosus muscle) were extracted with chloroform-methanol, according to the method of Folch et al. (1957, J. Biol. Chem. 226:592-596). Total fatty acids was esterified according to the method of AOAC Official Method 991.39 (1995) and then analyzed by gas 15 chromatography.

Expression levels of the bFGF gene (basic growth factor of fibroblasts) in intra-muscular fat and in muscle fibers: For these analyses, we begun by 20 manually separating the muscle fibers from the intramuscular fat, under a binocular. This separation to evaluate the expression of bFGF enabled us specifically in intra-muscular fat and in muscle fibers. Once the separation has been completed, total Transcriptionextracted and RT-PCR (Reverse RNA Polymerase Chain Reaction) analyses was performed for the bFGF gene. The RT-PCR analyses permitted us to quantify this gene's expression (i.e. the quantity of RNA expressed by this particular gene) and to verify if 30 there are any differences between normal and affected pigs.

Comprehension of the mechanisms involved in the development of steatosis at the cellular and tissular 15

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level: This aspect of the example permitted us to point out the mechanism of the development of steatosis.

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Measure of bound carnitine: In the preceding example, no significant difference was observed in the levels of total carnitine in muscle. However, a in significant increase was observed plasmatic carnitine in pigs affected by steatosis. These results gave us no information concerning the proportion of carnitine bound to fatty acids and free carnitine. This permitted us to verify if there is indeed a problem with the association of carnitine to long-chain fatty acids.

Determination of the levels of vitamin E in the muscles studied: In the previous example, we observed that steatosis-affected pigs accumulate more vitamin E in their liver than do normal pigs. It is therefore necessary to measure the levels of vitamin E in the ham muscles in order to verify if there is less vitamin E in affected muscles, which could entail a higher oxidative stress to these muscles.

Measure of the peroxydation levels of fatty acids: The decrease of certain fatty acids. (C15:1, C18:2 and C20:4) observed in the preceding example could be due to a higher peroxydation activity in affected muscles. In order to verify this, these levels of peroxydation in the semi-tendinosus muscle were measured according to the method of Witte et al. (1970, J. Food. Sci. 35:582-585).

Identification of new, easily metabolic or genetic factors: This section permitted us to identify other factors, such as fatty acids, proteins or genes which t permit us to rapidly

discriminate between affected and non-affected pigs, by way of simple fat tissue biopsies or blood samples.

Measure of the fatty acids present in red blood cells: This part of the example permitted us to identify if there are differences in the fatty acid profiles of affected pigs. The identification of differences in one or more fatty acids in red blood cells permitted us to use the blood of animals to determine their steatosis levels by simple gas thromatography analysis.

Identification of involved genes development of steatosis: To identify these genes, we have used a new molecular biology technique called "subtractive libraries". This technique has permitted us to compare two populations of messenger RNA (expression levels of a gene) in order to obtain clones of genes that are expressed strongly in one population (steatosis-affected pigs) and weakly, or not at all, in the other (normal pigs) and vice-versa. In order to help us achieve this aspect of the example, we have used two kits commercialized by CLONTECH: "PCR-Select Differential Screening Kit" and "PCR-Select cDNA Subtraction Kit". These analyses were performed on subcutaneous fat, intra-muscular fat and muscle fibers.

25 Results

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Results of the second experiment are summarized in tables 9 to 16 respectively.

TABLE 9
CARNITINE ANALYSIS IN DIFFERENT TISSUES

·		Nor	mal	Affe	cted	SEM	Р .		
Variable	Tissue	Fat	Lean	Fat	Lean	SEIVI .	Adipo- sity	Stea- tosis	A/St
Camitine (total) (nmoles/mg protein)	Muscle	58.11	57.12	62.06	55.20	3.35	0.217	0.747	0.354
Carnitine (free) (nmoles/mg protein)	Muscle	39.23	37.14	39.87	35.02	2.73	0.183	0.774	0.592
Carnitine (bound) (nmoles/mg protein)	Muscle	18.88	19.98	22.19	20.18	1.78	0.785	0.300	0.357
Carnitine (total) (umoles/liter)	Plasma	25.98	30.78	36.91	41.19	4.88	0.325	0.024	0.954
Camitine (free) (umoles/liter)	Plasma	16.98	21.32	25.33	25.18	2.64	0.400	101017	0.368
Carnitine (bound) (umoles/liter)	Plasma	9.00	9.47	11.58	16.01	2.56	0.312	0.063	0.412

⁵ legend: Fat, backfat \geq 22 mm; Lean, backfat \leq 19 mm; A/St, adiposity*steatosis; SEM, standard deviation/ \sqrt{n} (n=12); P, probability; significant when p<0.05 (shaded).

TABLE 10 VITAMIN E ANALYSIS IN DIFFERENT TISSUES

		Nor	mal	Affe	cted	SEM		Р	
Variable	Tissue	Fat	Lean	Fat ·	Lean	}	Adipo- sity	Stea- tosis	A/St
α-tocopherol (ug/g tissue)	liver	2.14	2.04	3.81 [']	3.55	0.147	0.244	0.000	0.594
γ-tocopherol (ug/g tissue)	liver	0.17	0.17	0.15	0.17	0.018	0.399	0.516	0.525
α-tocopherol (ug/g tissue)	muscle	0.86	0.96	1.16	1.05	0.078	0.975	0.022	0.215
y-tocopherol (ug/g tissue)	muscle	0.21	0.25	0.26	0.24	0.022	0.581	0.564	0.241
α-tocopherol (ug/g tissue)	muscle fiber	1.02	1.01	0.44	1.17	0.135	0:012	0.140	0.012
γ-tocopherol (ug/g tissue)	muscle fiber	0.20	0.22	0.12	0.21	0.020	0.015	0:047	0.115

5 Legend: Fat, backfat \geq 22 mm; Lean, backfat \leq 19 mm; A/St, adiposity*steatosis; SEM, standard deviation/ \sqrt{n} (n=12); P, probability; significant when p<0.05 (shaded). $0.000*,p \le 0.00001$.

TABLE 11

PEROXIDATION LEVELS OF INTRA-MUSCULAR FAT BY THE 10 THIOBARBITURIC ACID (TBA) METHOD (WHITE ZONE OF THE SEMI-TENDINOSUS MUSCLE)

	Nor	mal .	Affe	cted	SEM		Р	
Variable	Fat	Lean	Fat	Lean	JEIN	Adipo- sity	Stea- tosis	A/St
TBAJ0	0.144	0.155	0.128	0.179	0.018	0.051	0.924	0.180
TBAJ4	0.177	0.212	0.217	0.241	0.022	0.151	0.123	0.845
TBAJ9	0.201	0.221	0.318	0.288	0.391	0.891	0.015	0.489

TABLE 12
RED ZONE OF THE SEMI-TENDINOSUS MUSCLE

	Nor	mal	Affe	cted	SEM		P	
Variable	Fat	Lean	Fat	Lean	SLIVI	Adipo- sity	Stea- tosis	A/St
TBAJ0	0.242	0.220	0.164	0.188	0.032	0.743	0.147	0.254
TBAJ4	0.270	0.289	0.280	0.265	0.027	0.742	0.907	0.796
TBAJ9	0.295	0.289	0.342	0.340	0.033	0.791	0.076	0.836

5 Legend: Fat, backfat ≥22 mm; Lean, backfat ≤19 mm; A/St, adiposity*steatosis; SEM, standard deviation/\(\frac{1}{2}\) (n=12); TBA, thiobarbituric acid; J0, J4, J9, days 0, 4 and 9 respectively; P, probability; significant when p<0.05 (shaded).

TABLE 13
EXPRESSION LEVELS (RT-PCR) OF bFGF IN NORMAL AND
STEATOSIS-AFFECTED PIGS

	Normal		Affected		SEM	Ρ.		
Variable	Fat	Lean	Fat	Lean	SEIVI	Adipo- sity	Stea- tosis	A/St
bFGF muscle fibers (ng ratio)	0.790	0.956	0.572	0.606	0.13	0.403	0.022	0.582
bFGF intra- muscular fat (ng ratio)	1.259	1.175	1.671	2.135	0.24	0.386	0.003	0.214

Legend: Fat, backfat ≥22 mm; Lean, backfat ≤19 mm; A/St, adiposity*steatosis; SEM, standard deviation/√n (n=12); P, probability; significant when p<0.05 (shaded). The values in the table represent the quantity in ng calculated according to a standard curve. The relative values are standardized according to the pig with the highest level of mRNA expression.

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TABLE 14
ERYTHROCYTE FATTY ACID COMPOSITION (%) IN NORMAL AND
AFFECTED PIGS

	Normal Affected		cted	SEM	Р			
Fatty acid	Fat	Lean	Fat	Lean		Adiposity	Steatosis	A/St
C14:0	0.22	0.23	0.21	0.22	0.012	0.374	0.651	0.830
C15:0	0.16	0.17	0.13	0.14	0.015	0.444	0.062	0.640
C16:0	21.05	20.95	20.66	20.99	0.382	0.772	0.657	0.583
C16:1	0.56	0.60	0.55	0.55	0.022	0.361	0.227	0.449
C17:0	0,78	0.76	0.66	0.75	0.040	0.447	0.132	0.163
C17:1	0.18	0.18	0.18	0.19	0.013	0.868	0.937	0.847
C18:0	16.46	17.18	15.92	16.67	0.339	4 n0 0414	0.138	0.962
C18:1n9t	0.97	0.99	0.93	0.96	0.026	0.306	0.227	0.889
C18:1n9c	21.46	20.87	19.70	21.63	0.906	0.472	0.594	0.181
C18:1c11	0.87	0.92	0.80	0.86	0.021	产10:010元	£.10:003 S	0.647
C18:2n6c	31.38	30.91	34.31	30.73	1.306	0.137	0.311	0.251
C18:3n3	0.31	0.34	0.36	0.36	0.027	0.607	0.221	0.648
C20:0	0.13	0.11	0.11	0.13	0.007	0.526	0.838	¹⁴ 0.023
C20:1	0.22	0.20	0.22	0.19	0.017	0.179	0.556	0.697
C20:2	0.31	0.30	0.26	0.29	0.009	0.310	\b\0!003 €	÷ 0.016
C20:3n6	0.51	0.46	0.42	0.46	0.025	0.995	0.103	0.114
C20:4n6	4.31	4.70	4.47	4.74	0.187	0.088	0.610	0.765
C22:0	0.12	0.13	0.12	0.14	0.014	0.285	0.914	0.757

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Legend: Fat, backfat \geq 22 mm; Lean, backfat \leq 19 mm; A/St, adiposity*steatosis; SEM, standard deviation/ \sqrt{n} (n=12); P, probability; significant when p<0.05 (shaded).

TABLE 15
BACKFAT FATTY ACID COMPOSITION (%) IN NORMAL AND AFFECTED PIGS

	Noi	mal	Affe	cted	SEM	Р		
Fatty acid	Fat	Lean	Fat	Lean		Adiposity	Steatosis ·	A/St
C14:0	1.65	1.55	1.60	1.51	0.033	#0.008	0.227	0.86
C16:0	27.73	25.86	27.62	25.92	0.324	£ 0.000\$₩	0.954	0.792
C16:1	2.63	2.62	2.41	2.41	0.075	0.936	0.009	0.91
C17:0	0.28	0.29	0.25	0.29	0.013	0.101	0.167	0.45
C17:1	0.28	0.29	0.23	0.27	0.013	0.073	€ e0 035 3	0.28
C18:0	12.59	12.00	14.60	13.78	0.219	£0,003 3	30000 H	0.59
C18:1n9t	0.63	0.65	0.64	0.70	0.033	0.249	0.371	0.67
C18:1n9c	29.57	29.10	28.81	28.95	0.342	0.644	0.196	0.38
C18:1c11	1.73	1.74	1.61	1.65	0.034	0.525	160.004	0.67
C18:2n6t	0.13	0.14	0.12	0.13	0.003	0.005	0.029	0.21
C18:2n6c	19.39	22.23	18.81	21.00	0.425	0.000	此 0.044 经	0.45
C18:3n3	0.79	0.91	0.73	0.84	0.024	\$\$\0.00002;	£70.016	0.88
C20:0	0.19	0.15	0.21	0.18	0.011	0.005	0.050	0.66
C20:1	1.13	1.05	1.12	1.04	0.039	0.050	0.822	0.96
C20:2	0.64	0.69	0.62	0.66	0.014	0.003	0.054	0.87
C20:3n6	0.13	0.12	0.11	0.11	0.009	0.664	0.150	0.41
C20:3n3	0.13	0.14	0.12	0.12	0.004	640.043F	W# 0.0102 ;	0.25
C20:4n6	0.21	0.26	0.21	0.25	0.009	0.00003	0.527	0.47
C21:0	0.18	0.22	0.17	0.19	0.009	4,00.005.数	0.017	0.40

Legend: Fat, backfat ≥ 22 mm; Lean, backfat ≤ 19 mm; A/St, adiposity*steatosis; SEM, standard deviation/ \sqrt{n} (n=12); P, probability; significant when p<0.05 (shaded); 0.000*, p \leq 0.00001.

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TABLE 16
MUSCLE FATTY ACID COMPOSITION (%) IN NORMAL AND AFFECTED
PIGS

	Nor	mal	Affe	ected	SEM	Р		
Fatty acid	Fat	Lean	Fat	Lean		Adiposity	Steatosis	A/St
C14:0	1.47	1.41	1.67	1.47	0.040	0.002	.∵.0 0003:(∴	0.109
C16:0	28.76	27.74	30.70	28.55	0.313	? ⁷ 0.00001°.	0,0001	0.087
C16:1	3.10	3.10	3.23	2.87	0.144	0.233	0.753	0.227
C17:0	0.21	0.20	0.18	0.21	0.011	0.544	0.259	0.183
C17:1	0.23	0.24	0.20	0.23	0.013	0.087	0.133	0.337
C18:0	14.41	13.70	15.18	14.81	0.315	0.101	0.006	0.595
C18:1n9t	0.77	0.84	0.85	0.84	0.041	0.487	0.346	0.385
C18:1n9c	27.36	27.15	28.46	28.27	0.570	0.734	0.063	0.992
C18:1c11	2.03	2.11	1.97	1.95	0.063	0.657	0.093	0.459
C18:2n6c	17.82	19.22	14.40	17.23	0.565	育0,001%	҈0.00003 ⊊₩	0.226
C18:3n3	0.55	0.59	0.51	0.56	0.028	0.135	0.317	0.895
C20:0	0.15	0.12	0.18	0.16	0.011	10.024章性	ಪ್ಪ 0.002₹	0.976
C20:1	0.84	0.86	0.89	0.89	0.032	0.751	0.223	0.713
C20:2	0.42	0.46	0.40	0.45	0.015	∌0!004 ;;;	0.323	0.606
C20:3n6	0.25	0.28	0.16	0.20	0.018	0.058	11:10.00004	0.780
C20:4n6	1.52	1.87	0.90	1.20	0.122	35,0,013,	0.00001	0.849
C21:0	0.11	0.11	0.11	0.11	0.009	0.941	0.840	0.682

Legend: Fat, backfat \geq 22 mm; Lean, backfat \leq 19 mm; A/St, adiposity*steatosis; SEM, standard deviation/ \sqrt{n} (n=12); P, probability; significant when p<0.05 (shaded).

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conclusion, the present experiment In demonstrates clearly that several MSMF are correlated with the steatotic state in pigs. It has been determined that the muscular superoxide dismutase, and hepatic Vitamin E are correlated with the muscular In addition, it can be seen from the steatosis. present results that fatty acids have a direct relation with the muscular steatosis, as well in sub-cutaneous muscular samples. Also, from the RT-PCR as discrimination performed in fat or muscular samples, it was observed that EGF, IGF1R, IGF2, aFGF, FGF-2, TGFα, PDGF α , PDGF β , LPL, and the Leptin are each one good markers in determining the steatosis status of animals.

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Most particularly, the present invention shows that amplification of selected MSMF, it is to say the Leptin, FGF-2 and IGF1R are particularly accurates for identifying differential genetic expression in diagnosing the steatosis. The FGF-2 allows discrimination of steatotic pigs in 91.67 percent. Combination of factors makes possible to select non-steatotic from steatotic individuals in closed to 99 percents of the cases.

10 While the invention has been described in connection with specific embodiments thereof, it has been understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features herein before set forth, and as follows in the scope of the appended claims.

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WHAT IS CLAIMED IS:

- 1. A method for prognosis or diagnosis of muscular steatosis based on the level of muscular steatosis-modulating factor (MSMF) in a human or animal, which comprises the steps of:
 - a) measuring level of at least one MSMF in a biological sample of said human or animal, and
 - b) comparing the level of MSMF measured in the biological sample of said human or animal with the level of MSMF measured in a biological sample of a healthy human or animal, wherein a difference indicates predisposition or occurrence of steatosis.
- 2. A method according to claim 1, wherein said animal is selected from the group consisting of mammal and avian.
- 3. A method according to claim 1, wherein said animal is selected from the group consisting of porcine, bovine, ovine, caprine, chicken, turkey, horse, goat, canine, and feline.
- 4. A method according to claim 1, wherein said measuring of step a) is performed by identifying differential expression of MSMF gene.
- 5. A method according to claim 4, wherein said MSMF gene comprises a nucleic acid sequence selected from

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the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.

- 6. A method according to claim 1, wherein said MSMF is selected from the group consisting of growth hormone, growth factor, cytokine, growth factor receptor, growth hormone receptor, cytokine receptor, and lipid.
- 7. A method according to claim 1, wherein said MSMF is IGF1, IGF2, α FGF, FGF-2, ADRP, IGF1R, PDGF α , TGF β , TGF α , LPL, EGF, PDGF β , Leptin, superoxide dismutase, carnitine, creatine kinase, a vitamin, or a combination thereof.
- 8. A method according to claim 1, wherein said biological sample is blood, serum, plasma, a biopsy, fat, saliva, feces, or urine.
- 9. A method according to claim 1, wherein said measuring MSMF of step a) consists of measuring the level of at least one peptide, precursor, metabolite, or a messenger RNA of MSMF.
- 10. A method according to claim 9, wherein said messenger RNA is complementary to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.
- 11. A method according to claim 9, wherein said messenger RNA is corresponding to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.

A method for the treatment of muscular steatosis 12. in a human or an animal, which comprises regulating level substantially equivalent to that of a MSMF healthy human or a healthy animal.

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- A method according to claim 12, wherein said 13. regulation is performed by administrating an agonist of MSMF, an antagonist of MSMF, or a combination thereof.
- A method according to claim 13, wherein said agonist of MSMF is a MSMF.
- A method according to claim 13, wherein said 15. agonist of MSMF is a recombinant MSMF, a precursor of MSMF, a non-mature MSMF, an analog of MSMF, a purified MSMF, or a physiologically active fragment of at least one MSMF.
- A method according to claim 13, wherein said agonist of MSMF is an abzyme.
- A method according to claim 13, wherein said 17. antagonist of MSMF is an inhibitor of MSMF.
- A method according to claim 13, wherein said antagonist of MSMF is an abzyme.
- 19. A method according to claim 13, wherein said antagonist of MSMF is selected from the consisting of an antibody, an anti-MSMF messenger RNA, a MSMF RNA ligand, a MSMF-specific antisense primer, an anti-MSMF receptor, and a mutant MSMF.

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- 20. A method according to claim 19, wherein said anti-MSMF messenger RNA is complementary to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.
- 21. A method according to claim 19, wherein said anti-MSMF messenger RNA is corresponding to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.
- 22. A method according to claim 13, wherein said agonist of MSMF, antagonist of MSMF, or combination thereof is administered by introducing at least one expression vector into the human or the animal.
- 23. A method according to claim 22, wherein the expression vector is introduced into at least one cell, and said cell is introduced into the human or the animal.
- 24. A method according to claim 13, wherein the agonist of MSMF or the antagonist of MSMF is administered systemically, orally, or intravenously, using an implant or a slow delivery system.
- 25. A method for causing muscular steatosis in an animal for increasing fat content, which comprises the step of administrating to said animal a sufficient amount of at least one agonist of MSMF, an antagonist of MSMF, or a combination thereof, for deregulating MSMF in said animal to a level different from the level of MSMF of a healthy animal.

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- 26. A method according to claim 25, wherein said agonist of MSMF is a MSMF.
- 27. A method according to claim 25, wherein said agonist of MSMF is selected from the group consisting of recombinant MSMF, precursor of MSMF, non-mature MSMF, analog of MSMF, purified MSMF, and a physiologically active fragment of a MSMF.
- 28. A method according to claim 27, wherein said agonist of MSMF is an abzyme.
- 29. A method according to claim 25, wherein said antagonist of MSMF is a MSMF.
- 30. A method according to claim 25, wherein said antagonist of MSMF is an abzyme.
- 31. A method according to claim 25, wherein said antagonist is selected from the group consisting of an antibody, an anti-MSMF messenger RNA, a MSMF RNA ligand, a MSMF-specific antisense primer, an anti-MSMF receptor, a synthetic antisenses, a natural antisenses, and a mutant MSMF.
- 32. A method according to claim 31, wherein said anti-MSMF messenger RNA is complementary to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.
- 33. A method according to claim 31, wherein said anti-MSMF messenger RNA is corresponding to a nucleic

acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.

- 34. A method according to claim 25, wherein said agonist, antagonist, or combination thereof is administered by introducing at least one expression vector into the animal.
- 35. A method according to claim 34, wherein said expression vector is introduced into at least one cell, and said cell is introduced into said animal.
- 36. A method according to claim 25, wherein an agonist or antagonist is administered systemically, orally, or intravenously, using an implant or a slow delivery system.
- 37. A method according to claim 35, wherein said muscular steatosis is caused in said animal by administrating an agonist of MSMF, an antagonist of MSMF, or a combination thereof.
- 38. A compound of the group of MSMF for the treatment of muscular steatosis in a human or an animal patient.
- 39. A compound according to claim 38, which is selected from the group consisting of an agonist of MSMF and an antagonist of MSMF, or a combination thereof.
- 40. A compound according to claim 39, wherein said agonist of MSMF is a MSMF.

- 41. A compound according to claim 39, wherein said agonist is a recombinant MSMF, a precursor of MSMF, a non-mature MSMF, an analog of MSMF, a purified MSMF, or a physiologically active fragment of a MSMF.
- 42. A compound according to claim 39, wherein said agonist of MSMF is an abzyme.
- 43. A compound according to claim 39, wherein said antagonist of MSMF is a MSMF.
- 44. A compound according to claim 39, wherein said antagonist of MSMF is an abzyme.
- 45. A compound according to claim 39, wherein said antagonist of MSMF is selected from the group consisting of an antibody, an anti-MSMF messenger RNA, a MSMF RNA ligand, a MSMF-specific antisense primer, an anti-MSMF receptor, and a mutant MSMF.
- 46. A method according to claim 45, wherein said anti-MSMF messenger RNA is complementary to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.
- 47. A method according to claim 45, wherein said anti-MSMF messenger RNA is corresponding to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.
- 48. A compound according to claim 39, wherein said agonist of MSMF, antagonist of MSMF or combination

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thereof is administered by introducing at least one expression vector into the human or the animal patient.

- 49. A compound according to claim 48, wherein said expression vector is introduced into at least one cell, and said cell is introduced into said human or said animal patient.
- 50. A compound according to claim 39, wherein said agonist of MSMF or antagonist of MSMF is administered systemically, orally, or intravenously, using an implant or a slow delivery system.
- 51. A compound of the group of MSMF for causing muscular steatosis in an animal.
- 52. A compound according to claim 51, which is selected from the group consisting of an agonist of MSMF and an antagonist of MSMF, or a combination thereof.
- 53. A compound according to claim 51, wherein said agonist of MSMF is a MSMF.
- 54. A compound according to claim 52, wherein said agonist of MSMF is a recombinant MSMF, a precursor of MSMF, a non-mature MSMF, an analog of MSMF, a purified MSMF, or a physiologically active fragment of a MSMF.
- 55. A compound according to claim 52, wherein said agonist of MSMF is an abzyme.

- A compound according to claim 52, wherein said 56. antagonist of MSMF is a MSMF.
- A compound according to claim 52, wherein said antagonist of MSMF is an abzyme.
- A compound according to claim 52, wherein said 58. antagonist of MSMF is selected from the consisting of an antibody, an anti-MSMF messenger RNA, a MSMF RNA ligand, a MSMF-specific antisense primer, an anti-MSMF receptor, and a mutant MSMF.
- 59. A compound according to claim 58, wherein said anti-MSMF messenger RNA is complementary to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.
- A compound according to claim 58, wherein said 60. anti-MSMF messenger RNA is corresponding to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:305 or a fragment thereof.
- 61. A compound according to claim 52, wherein said agonist of MSMF, antagonist of MSMF or combination thereof is administered by introducing at least one expression vector into the animal.
- 62. A compound according to claim 61, wherein said expression vector is introduced into at least one cell, and said cell is introduced into said animal.
- A compound according to claim 52, wherein said 63. agonist of MSMF or antagonist of MSMF is administered

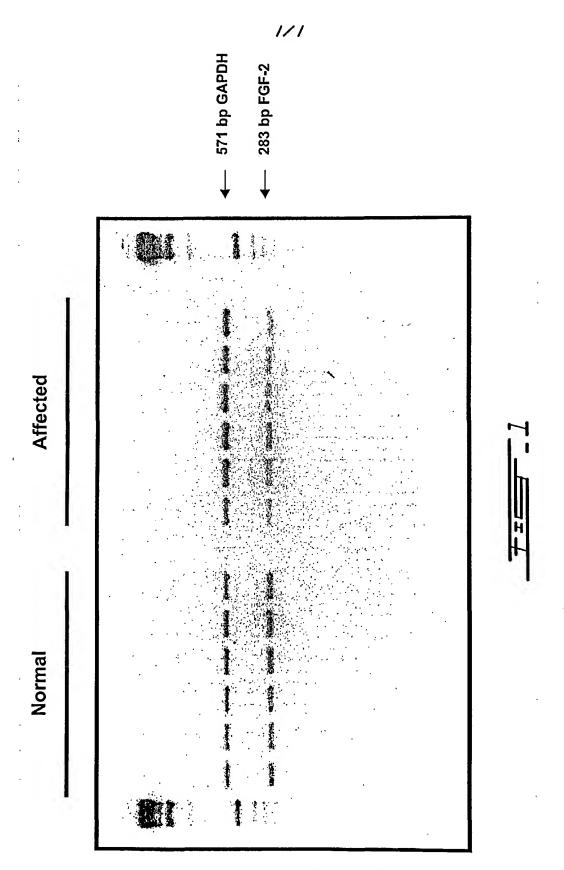
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systemically, orally, or intravenously, using ar implant or a slow delivery system.

- 64. Use of a compound as defined in any one of claims 38 to 50 for treating muscular steatosis.
- 65. Use of a compound as defined in any one of claims 51 to 63 for causing muscular steatosis.
- 66. Use of a compound of the group of MSMF in the manufacture of a medicament for treating muscular steatosis.
- 67. Use of a compound as defined in any one of claims 38 to 50 in the manufacture of a medicament for treating muscular steatosis.
- 68. Use of a compound of the group of MSMF in the manufacture of a medicament for causing muscular steatosis.
- 69. Use of a compound as defined in any one of claims 51 to 63 in the manufacture of a medicament for inducing muscular steatosis.
- 70. A pharmaceutical composition comprising a compound as defined in any one of claims 38 to 50 in association with a pharmaceutical acceptable carrier.
- 71. A pharmaceutical composition for use in the treatment of muscular steatosis comprising a therapeutically acceptable and effective amount of a

compound of the group of MSMF in association with a pharmaceutically acceptable carrier.

72. A pharmaceutical composition for use in causing muscular steatosis comprising a therapeutically acceptable and effective amount of a compound of the group of MSMF in association with a pharmaceutically acceptable carrier.



SUBSTITUTE SHEET (RULE 26)

SEOUENCE LISTING

<110> Sa Majesté la Reine du Chef du Canada PALIN, Marie-France POMAR, Candido GARIÉPY, Claude <120> Steatosis-modulating factors and uses thereof <130> 14654-2PCT <140> 60/197936 <141> 2000-04-17 <150> 60/197936 <151> 2000-04-17 <160> 305 <170> FastSEQ for Windows Version 4.0 <210> 1 <211> 792 <212> DNA <213> Artificial Sequence <220> <223> Artificial sequence Muscular steatosis Porcine <400> 1 cctttgcgnc gaccgttgac tattctctac aaaccacaaa gacattggaa cactatacct 60 attattegge geatgagetg gagteetagg cacageteta ageeteetta ttegageega 120 gctgggccag ccaggcaacc ttctaggtaa cgaccacatc tacaacgtta tcgtcacagc 180. ccatgcattt gtaataatct tcttcatagt aatacccatc ataatcggag gctttggcaa 240 ctgactagtt cccctaataa tcggtgccc cgatatggcg tttccccgca taaacaacat 300 aagettetga etettacete eeteteeet aeteetgete geatetgeta tagtggagge 360 cggagcagga acaggttgaa cagtctaccc tcccttagca gggaactact cccaccctgg 420 agecteegta gacetaacca tetteteett acacetagea ggtgteteet etatettagg 480 ggccatcaat ttcatcacaa caattatcaa tataaaaccc cctgacccta cctgcccggg 540 eggeegeteg aageegaatt etgeagatat ceateacaet ggeggeeget egageatgea 600 tctaqaqqqc ccaattcqcc tataqtqaqt cqtattacaa ttcactqqcc qtcqttttac 660 aacgtcgtga ctgggaaaac cctggcgtta cccaacttaa tcgccttgca gacattcccc 720 tttcgccaag ctggcgtaat acgaanangc ccgnaccgat cggccttcca acagttgcgc 780 aacctggaat gg 792 <210> 2 <211> 807 <212> DNA <213> Artificial Sequence <220> <223> Artificial sequence Muscular steatosis Porcine <400> 2 actgatgttc gccgaccgtt gactattctc tacaaaccca aagacattgg aacactatac 60 ctattattcg gcgcatgagc tggagtccta ggcacagctc taagcctcct tattcgagcc 120

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Muscular steatosis Porcine

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Porcine

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Muscular steatosis Porcine

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Porcine

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Porcine

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Muscular steatosis Porcine

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naaageegaa ttecacacae tgeggnenta etagtggate ceatgetegg taccaagett
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ggcgtaanca ttggtcntaa nctgattcct gtgtgaaatt gtntccgctt cacaatntcc
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ncaccaacat acgaacccgg aagcattaaa ntgtaaaagc ctggggtgcc taatgagtga
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tgactttggc tatgaaggtg atatcttcac caactttcac tgttcctcct tgaggttttt
                                                                           180
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Porcine

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Muscular steatosis Porcine

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Muscular steatosis Porcine

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gcagcatatg aattaaaaaa aaannnnntt aaaaaaaaag cttgngncct gccggggcgg
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gccgntnnaa aaccnaaatt ccagccactt ggggggccgt tactaagggg anccaaactt
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cgngcccaac cnttgggtaa atcatnggca ananctggtt cccctgggng naaantgntn
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Muscular steatosis Porcine

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                                                                         600
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                                                                         360
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ctctgaacat aacaaagcaa caacatcccg gtgtttactt atcagaggag acaatgcttt tctttcanag aaaatgtncc	tggattacct gtgatttctt agtaagagat agttcagtgc agtggtgtaa ccttccttct gaattttgaa taaaccttat tgcccgggcg cccaanctng	tccctaacac atttttgctg acatctaaca agtgaacaag gagtttttac cacttgaata tttagnacca gcccgtcaaa	tagitittec ttactttgac attcactcaa aaaagcagca ataagttgat aaangttnga gcattccttc agccgaattc	aatctactaa aattctctct gactaggcac taataaagga gccttactgc agctttgaat catagcncat cagnanactt	tttatttata ctccaatccc ctgacaataa ctgngttttt acctttgaat aaaatcctcc aaatntgtnc ggcggccgtt	180 240 300 360 420 480 540 600 660 713
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                                                                       420
                                                                       480
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      Porcine
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240

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                                                                         480
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                                                                         240
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gttattaaaa gggctggtag ggctccggta agtggtcatg ggcttgggtt tactatgtgg
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      Porcine
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	scular steato rcine	sis				
tgaaggtg acattcat tgaatatc aattacag tgaccatc ggctgaat ttatagac tgaaatgt atgctgca tttttgcc	ga acggactgct gt gaatgacctt gt gaatgacctt ac tggtgagctt ct aggaaaagte cc ccatgatcga cc taaaagtate cc tgtaaaacaa cg ctggaagaac gg acacattgaa tc catgttggga nc ttcctttcte gc ataatctngg	gtgttcagtg gtgcggatct atggtgacgg ttgcaagttt atttatactg taccgctgct cacttgctga tgtgatgctt cgggatgctt agtctttaat	gttctagtga ataaaggtca cttgcctgga atggaggaca gctgttatga ggtggcatgg ctgaccatac tttttactgc aggatgacag tgaagtaatt gggaanaaaa	tcagtcagtc caatcatgca taaatttgtt caaagacatg cggcagtatt ttgtgctctg aaatccaaac taggaaagga caaaattgat tcacattggc aagtggtact	cacgcccaca gtgactgcgg cgtgtctatg attatgtgta caggccgtga atatttggtg tttcagactc tccaaacagg tcatgaaagt cccttacata tagcangctt	60 120 180 240 300 360 420 480 540 600 660 720 780

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      Porcine
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agggtccctt cgganaccca ggggccgatg gcatcggcac anagcgcccg ggttcacagt
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ctcctgctca atggtctctt tggtgggcag ggtgttcttc tcctgcgtct ccgtcttctt
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cagettggcc ttgtcgaanc tgttgatttc gcccatgtcc ggcttgtctg ccattttctt
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ataacaatcc gagaagttcc gctgaaagcc caaggtgctg ctactngcac tcgcctngct
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gcancaagan accccgcgt acctgcccgg gcggccgttc aaacccaatt ncacanactg
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geggeegine tantggatee caacteggne caaactiggn gnaateatgg catanetgtt
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nctqnqnqaa aatgntatcc nntnanattn cncccannta cnanccggaa ncntnaaann
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010 7377
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ncttatnggg gttgcctgat gggaggggga gggagtggga gggatcggga gcttgggctt 360 atnagnenca acgtanaata natttacnng gganatcccg ntgaanagen ttganaactn 420
tgntananac tcanggntgn ccanaaaaaa nggggggga aaaaactgnn nttgnnangn 480
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ncnncactgg ggggcngtnc tagggga 56

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cctggcttgn aaatctattc taactngtgt ctgataagcc caagctcccg ggccctccca
                                                                       180
                                                                       240
ctcctnccc cttncatcan gcagccacaa gnntcttctn canntncatg attttctttn
                                                                       300
ctgaggagat gctcatttgn gctggatatt agatnncagt tataagtgat attcatatgc
                                                                       360
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ggtgcttgcn aatggcatta ngtcattctt tttttatggc tgaccncnta ttccattggn
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                                                                       480
gtatatanac cacctctccg aatccagncc atntggnnat gnacattttg gntnncctcc
atgtgcctgg nntntngtna atantcccca gttccttgcc cgggaggccc gttttaaagc
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cnanttecag geceacttge nggengttee tantgggate ccaaaceten ggngecaaaa
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cnanattecn nnecgeatta entaaceegg aaanatnaaa ggngnaacee etgggggggg
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agtgttctta tactaaaaat aaaagcaaaa atatgagatc atgcactaga gacaaaagct
                                                                       240
acaagtgaag agttatagca aaaaacattg caaaaaataa aaatgttcaa gtattgcaag
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aggagagggg aagattgaaa tgaaacagtt tcaggataaa tcaattatat ttactactct
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aggagggaca ctgacataat tgnctttgcc attaatactt tatgctgtta aaatacacac
                                                                       420
tattatccat ttagaggaca gataccattt ccttggggca cctctcatat tacaaggatg
                                                                       480
gaataaaatt cactgnttac cttgactata aataaatgat aatactttag gagaaatatt
                                                                       540
cgcaaaagca ggccagtgtg atgccctgga atagtaagaa ccttggtgtt aggaaactgg
                                                                       600
gtctagtcct attattttat atcattggct gtgaaagata cttcccggg tcctgccng
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      Porcine
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tacagetgtg cactcaatce ggatecatct ettgagtete ggeeeetttt aaagaaceta
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agcagaataa gcataggaca ccccctcccc aaaaattgct agacctgccc atcatttctc
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                                                                       480
gcagcatcat ctgtttgaaa atataagcaa ttccctcatc taattatagg aaggatttga
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ggttcattaa cattgccaag gcagagaatc cagtgtgcaa cttcagagct ttgcttggtg
gcctgtgttc tcagtttcct tttattgctt tctcaaggtc tccaagactt tttcctgaaa
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                                                                       660
tcataaaatt aagttttcct tncaaataga cacgtgagat agaagaaagg aaatgactac
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agtgtaaact attttatttc tttgggaatt ggttaagaaa aaaaagatta gaaggatggt
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    Porcine
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cagettteaa cattgaaaaa aattteaagt taaacaacaa aaatgteagt gtatetaaaa
                                                                       420
aagacttatt gagaaagcaa gcnaaaaaaa aaaaaaaaaa aaaaagtcct gcccggcggc
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                                                                       600
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      Porcine
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gacatttggg ttggcacgat accacgtttc ctttacaatt ctatncttca attnggaaat
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                                                                       615
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 gtaggacgat catcacccc atgtcagaaa acacaacctc ttccaaataa agaatatgac
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        Porcine
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Muscular steatosis

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Porcine

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 atcogotoac aattocacac aacatacgag coggaagcat aaagtgtaaa gootggggtg
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                                                                       240
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      Porcine
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      Porcine
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                                                                       720
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      Porcine
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						•
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	ficial seque ular steator ine					
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                                                                       180
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gaaggtgggt gaggccactg agacagcgct caccaccctg gtggagaaga tgaacgtatt
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                                                                       300
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tegecageta atgaagaagg aatteacact ggagttetee egggacagaa agtecatgte
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cgtctactgc tctccagcta aatcccgggc tgctgtgggc aacaagatgt ttgtcaaggg
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negeteaage egaatteeag cacaetggeg gnegttacta gtggateega geteggtace
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gtcccgtcta ctgctctcca gctaaatccc gggctgctgt gggcaacaag atgtttgtca
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aataatctcc cacattgtaa cctactattc aggtaaaaaa gaaccatttg gatatatagg
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tgctattccc actggagtaa aagtatttag ttgattagct accctgcacg gcggcaatat
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	ficial seque llar steatos ine					
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                                                                            300
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                                                                            420
                                                                            480
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gaattetgea gatateeate acaetggegg negetegage atgeatetag agggeeaatt
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                                                                            240
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      Porcine
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					•	
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Porcine

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. 110/122

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	ficial sequ ular steato ine			·		
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Porcine

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atgtttactc aacctttggt caatacttac gctgtagctg gttacaatgc taccctgaac
                                                                         300
tgcagtgtga gaggaaatcc taagcccaaa atcacctgga tgaaaaacaa agtgactatt
                                                                         360
aaggatgacc caagatacag gatgttcagc aaccaagggg tctgtaccca gctgtccccc
                                                                         420
                                                                         480
cactgtggga aggaacttct ggaagtgatc cattgagctg tcagagaaga catcagggtg
attttcatag ataaacttct ccaccctcat ttgccgtagt ttgaacatct tagagcccg
                                                                         540
gttagtgagc agcgacaact cctncaacat cacatccctt gggacactga tcttcttgcc
                                                                         600
caggiticagg cetgagetet cetgiceace incagtgagt tecatgatea gettgetggg
                                                                         660
atttcctctt cttgtttggg gctggggtcc cctgaaaacc ggcatttgtg gaagtgggtg
                                                                         720
gagccggctg ctcaggcggt ggtcaactnt tcctggggtc caacggcttt ggangacacc
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                                                                         814
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      Porcine
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                                                                         180
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                                                                         300
agagetteat eggagaatte atggagagee tgeaggacee agacetgaae gtgegeeggg
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                                                                         420
                                                                         480
tggatggcat cctgcccctg ctctaccagg agaccaagat ccaccgggac ctcatccgan
                                                                         540
aggtggagat ggggcctttt aagcacacag tggacgatgg gctggatgtg aagaaggcgg
cetttqaatq catgtacetq ceqqeqqeeq etegaaagce gaatteagca caetggegge
                                                                         600
cgttactagt ggatccgagc tcggtaccaa gcttggcgta atcatggcat agctggttcc
                                                                         660
tgtgtgaaat tgtatnccgt tacaattnca cacaacatac gancccggaa cntaaagtgt
                                                                         720
aaagectggg gtgcctaatg agtgagetaa ctcacattaa ttgcgttgcg ctnactggcc
                                                                         780
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<211> 825
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<220>
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      Muscular steatosis
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<210> 280 <211> 832 <212> DNA <213> Artificial Seque	ence				
<220> <223> Artificial seque Muscular steatos Porcine					
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       Porcine
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 gatggtaaac tctaactcct cctgaagttt ctgagcaaat actggggggg ttcttttctg
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                                                                        240
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 tgaggagggc atcancaaag gcaaagganc cctcggnagc tgtantttca atcacttntg
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 achogquec caaggeentq gentecaget ntttttqqaa qqattntttt qqcachtqca
                                                                        360
                                                                        420
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 gttcacaaaa nnttaagtnt cntnaaaact ggacntgaac acggttcggg nantccaact
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                                                                        540
 cccgggcggc ctntaaaanc cnattccanc acantggggc cgtntntagg ggatccnatc
                                                                        600
 tnggcncaan tntnggngaa antnngnata tatntgtccc tgtganaaan tgntatccnc
                                                                        660
 tcanaattca cacaatatan gaccengaac cataagtgaa accenggggc cetaagaggg
                                                                        720
                                                                        780
 actneceana ttanngggnn geentantge ettttneagg ggaaaaentg tneneenett
                                                                        801
 tattaaaaat cnccccccn g
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       Porcine
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 accaaggaag ggaaacacca agggtcgctg cacataaaaa tgccacctca tccctgatgc
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                                                                         300
                                                                        360
 gggtggggga gcagggagtg agtgagtgat ggtggagtga aacaagagca nagaaaaggg
 gctgggcagt tcagacctga gtcccaagct ccccactct ctggcccctg atttgccatg
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 ccccaatctt gtgagccacc cacaagccnc cagctacctn caggtgaaag ccanaaatga
                                                                         480
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                                                                        540
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                                                                        600
 aaaactttcc cnancccttn acctatnaca ngaagcnanc tttgtgncct nntgaaggnn
                                                                         660
 naccttnang ggttaattaa naaccttcac cctcctaagt tgccaatttt gaaaaatctt
                                                                        720
 ccctttccgg atacaggntg nngaaantgn ggctttgaca ccaggttnaa agaccttncc
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. <220>
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       Muscular steatosis
       Porcine
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catteacacg cacacattea etcaggegeg egegeacgea cacacacaca ecceagagee
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                                                                         180
accaaggaag ggaaacacca agggtcgctg cacataaaaa tgccacctca tccctgatgc
                                                                         240
acgcatgttc tcccaaggcc acgctcacac gacacacatt ataagcactt tgcctgattc
                                                                         300
acticactqqq totqtotttt gtgggaagga gaggaggaat toatcaagot otoctoccca
qqqtqqqqqa qcaqqqaqtg agtgagtgat ggtggagtga aacaagagca nagaaaaggg
                                                                         360
getgggeagt teagacetga gteceaaget ecceactet etggeeetg atttgeeatg
                                                                         420
ccccaatctt gtgagccacc cacaagccnc cagctacctn caggtgaaag ccanaaatga
                                                                         480
cggcttncac cttggcaccc caaanttgan gncanncagc aacccggggt tagaatgttt
                                                                         540
                                                                         600
ggaggttctn anaacnettt engetaaatn ateceettgg attaactngg etgtgeenta
aaaactttcc cnancccttn acctatnaca ngaagcnanc tttgtgncct nntgaaggnn
                                                                         660
                                                                         720
naccttnang ggttaattaa naaccttcac cctcctaagt tgccaatttt gaaaaatctt
ccctttccgg atacaggntg nngaaantgn ggctttgaca ccaggttnaa agaccttncc
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       Muscular steatosis
       Porcine
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                                                                         120
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 tgtagtaagg tgggaacett cgcctgcggt agggccggcg ctgttgggcc tgccttcggg
                                                                         180
                                                                         240
 agcactetet gatecetegt tetttteece acteteacta ttetggtaat tetgetggta
                                                                         300
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attactgcct tgactggaac tccaccaggg cctgtaacgt ttgctgcctc cgaaccettt tntccttnac accatcaaac tccacagtnt ttccatctcc tacactgcna aggtacctgc
                                                                         360
                                                                         420
 ccgggcggcc gttcaaaagc cgaattccag nacactgncg ccgtnctagg ggatccaanc
                                                                         480
                                                                         540
 togtacaact tgggcnaanc atgggcatan ctgtccctgt gaaaaattgg tttccgctca
 cattcccaca aaatnncaaa cccggaanna ttaaagtgta aaccctgggg ggcccnaatg
                                                                         600
 agtnanctnn actcacntta aattgnnttt ncnctncagt gcccnttttc aantcgnaaa
                                                                         660
                                                                         720
 aacctgtngg gcccanctgn tttaaanaat tggcccaacn ccncggngaa naggcntttg
 ctnttggngc ccttttccnt ttcctnggna naaaannntc nntgccctgg gncnttcggt
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       Porcine
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                                                                          120
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 agctgtggct tcatgaggtg cagtaacaaa gattgtttcc ccactgattc tattcatgta
                                                                          180
 gatgcaggta cetgceeggg eggcegeteg aaagccgaat tecagcacac tggeggeegt
                                                                          240
 tactagtgga tecgageteg gtaccaaget tggegtaate atggteatag etgttteetg
                                                                          300
 tgtgaaattg ttatccgctc acaattccac acaacatacg agccggaagc ataaagtgta
                                                                         360
 aageetgggg tgeetaatga gtgagetaac teacattaat tgegttgege teactgeeeg
                                                                          420
```

.

					•
ctttccagtc gggaaacctg gaggcgggtt gcgtattggg tcgttcggct gcggcgaac					480 540 559
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                                                                       120
aacteggnea tgatggnetn cateenenen agggeeaaga tgaengteae cangggtget
                                                                       180
geggangaag geggatggee atgtteatet ceagentetg eagentgtee acaatganga
                                                                       240
cnnagatetg atggtgggag taacgcaten geatggacae anteaeggtg aaaatgacen
                                                                       300
tgatgactaa ggcggncatg taggacgtgc gtgccatnca catgctcncg aancggtagt
                                                                       360
gctcccgga caccangtnt ccnaggaagn ccttgttntc ctcgttctnc gccatgccct
                                                                       420
                                                                       480
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geggteecen aaacactgga tteetegtgg agteeagggg tgacccacca tgacanggat
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                                                                       600
                                                                       660
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tttntaaggg cccaattacn cctattatga gtnctattan nnattcactt ggccgtcntt
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                                                                       841
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      Muscular steatosis
      Porcine
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ctggctnccc tgccatcaat ggccacagcc atacagatga cgaggagaca ccaggcgagt
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                                                                       240
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ctgangtggc agcagatagc cctgccccat gatggtcagc tctgcctccc tattctgtca
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                                                                       360
                                                                       420
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                                                                       480
tgngatctgg gtctgaacaa cactcatgat tcccagagac catatccaga tgtcnctgcc
                                                                       540
cacaccetgg teaggacett eteaaggegg enneactgna tgtetgattt catnetaane
                                                                       600
catcettgca tttactaatt cttgtntcct tgcttctccc ttccaatgaa agganttcan
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                                                                       720
                                                                       780
gateceanet ngnneceaag ettngnngtt annaanggtn atanntgnnt netggngnga
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qtcctqtccc acgagaacac tqqcgatgtg gctgtgtgga caqttqqtqq qaaaacggtg
                                                                       180
gccagetetg accettteca ggccaetege cagggcegea ageaectgee egggeggeeg
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ctcgaaagcc gaattctgca gatatccatc acactggcgg ccgctcgagc atgcatctag
                                                                       300
agggcccaat togccctata qtqagtcgta ttacaattca ctqqccqtcq ttttacaacg
                                                                       360
tegtgactgg gaaaaccetg geegttacce aacttaateg cettgeagea catececett
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(19) World Intellectual Property Organization International Bureau



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17 April 2000 (17.04.2000) U

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(75) Inventors/Applicants (for US only): PALIN, Marie-France [CA/CA]; 1055, Principale Sud, Waterville, Québec J0B 3H0 (CA). POMAR, Candido [CA/CA]; 320, chemin Biron, Fleurimont, Québec J1G 5E4 (CA). GARIÉPY, Claude [CA/CA]; 278, rue du Golf, Mont St-Hilaire, Québec J3H 1Z3 (CA).

- (74) Agents: CÔTÉ, France et al.; Swabey Ogilvy Renault, Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

with international search report

(88) Date of publication of the international search report:
6 September 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

'079287 A

(54) Title: STEATOSIS-MODULATING FACTORS AND USES THEREOF

(57) Abstract: The present invention relates to a method of modulating the muscular steatosis-modulating factors (MSMF). The determination of concentrations of the MSMF is described for the establishment of the steatotic state in individuals. Also, is disclosed a method of selecting individuals to serve as founders of animal lineages. The present method involved too the treatment of human and animals with agonists or antagonists of MSMF depending of the effects desired.

INTERNATIONAL SEARCH REPORT

Internat pplication No PCT/CA 01/00509

			7 00303
A. CLASSI IPC 7	GO1N33/74 C12Q1/68 A61K38/1	17 A61K39/395	
According to	o International Patent Classification (IPC) or to both national classific	allon and IPC	
B. FIELDS	SEARCHED		
Minimum do IPC 7	ocumentation searched (classification system followed by classification GO1N C12Q A61K	on symbols)	
Documenta	tion searched other than minimum documentation to the extent that s	such documents are included in the fields so	earched
Electronic d	lata base consulted during the international search (name of data ba	se and, where practical, search terms used)
EPO-In	ternal, WPI Data, PAJ, CHEM ABS Data	a, BIOSIS, MEDLINE	
C. DOCUM	ENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the rel	evant passages	Relevant to daim No.
A	WO 99 23493 A (UNIV ROCKEFELLER) 14 May 1999 (1999-05-14) the whole document		1
A	US 4 929 600 A (COGBURN LARRY A) 29 May 1990 (1990-05-29) abstract		1
A	WO 99 67631 A (DOYLE JOHN CONAN) 29 December 1999 (1999-12-29) abstract	·	1
Furt	her documents are listed in the continuation of box C.	X Patent family members are listed	In annex.
* Special ca	stegories of cited documents :	*T* later document published after the Inte	rnational filing date
	ant defining the general state of the art which is not lered to be of particular relevance	or priority date and not in conflict with cited to understand the principle or the	the application but sory underlying the
'E' earlier	document but published on or after the International	invention "X" document of particular relevance; the of	
filing of	ent which may throw doubts on priority claim(s) or	cannot be considered novel or cannot involve an inventive step when the do	be considered to current is taken alone
citation	is cited to establish the publication date of another n or other special reason (as specified)	"Y" document of particular relevance; the cannot be considered to involve an in	ventive step when the
	ent referring to an oral disclosure, use, exhibition or means	document is combined with one or mo ments, such combination being obvior	ore other such docu— us to a person skilled
	ent published prior to the international filling date but han the priority date claimed	in the art. *&* document member of the same patent	family
Date of the	actual completion of the international search	Date of mailing of the international sea	arch report
7	May 2002	17/05/2002	
Name and r	mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2	Authorized officer	
	NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,	, Managa A	
I	Fax: (+31-70) 340-3016	' Moreno, C	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 38-45, 48-72

Present claims 38-45 and 48-72 relate to compounds defined by reference to a desirable characteristic or property, namely being muscular steatosis-modulating factors.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/compound/method/apparatus by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Internat pplication No PCT/CA 01/00509

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 9923493	A	14-05-1999	WO	9923493 A1	14-05-1999
US 4929600	A	29-05-1990	AU	3360989 A	05-10-1989
			EP	0358754 A1	21-03-1990
		•	JP	2503987 T	22-11-1990
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